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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,321,256**, on October 16, 2000, by **PROCYON BIOPHARMA INC**, for "Pharmaceutical Preparations and Methods for Inhibiting Tumours".

L. Régimbal

Agent certificateur/Certifying Officer,

October 9, 2001

Date

Canada

(CIPO 68)
01-12-00

OPIC CIPO

PHARMACEUTICAL PREPARATIONS AND METHODS FOR INHIBITING TUMOURS

FIELD OF THE INVENTION

The present invention relates to pharmaceutical preparations for use as tumour suppressive agents for tumours arising from cancers such as prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial and ovarian cancers, and benign prostate hyperplasia (BPH).

BACKGROUND OF THE INVENTION

The prostate gland, which is found exclusively in male mammals, produces several components of semen and blood and several regulatory peptides. The prostate gland comprises stroma and epithelium cells, the latter group consisting of columnar secretory cells and basal nonsecretory cells. A proliferation of these basal cells as well as stroma cells gives rise to benign prostatic hyperplasia (BPH), which is one common prostate disease. Another common prostate disease is prostatic adenocarcinoma (CaP), which is the most common of the fatal pathophysiological prostate cancers, and involves a malignant transformation of epithelial cells in the peripheral region of the prostate gland. Prostatic adenocarcinoma and benign prostatic hyperplasia are two common prostate diseases which have a high rate of incidence in the aging human male population. Approximately one out of every four males above the age of 55 suffers from a prostate disease of some form or another. Prostate cancer is the second most common cause of cancer related death in elderly men, with approximately 96,000 cases diagnosed and about 26,000 deaths reported annually in the United States.

Studies of the various substances synthesized and secreted by normal, benign and cancerous prostates carried out

in order to gain an understanding of the pathogenesis of the various prostate diseases reveal that certain of these substances may be used as immunohistochemical tumour markers in the diagnosis of prostate disease. The three predominant proteins or peptides secreted by a normal prostate gland are: (1) Prostatic Acid Phosphatase (PAP); (2) Prostate Specific Antigen (PSA); and, (3) Prostate Secretary Protein of 94 amino acids (PSP^{94}), which is also known as Prostatic Inhibin Peptide (PIP), Human Seminal Plasma Inhibin (HSPI), or β-microseminoprotein (β-MSP), and which is hereinafter referred to as PSP^{94}.

PSP^{94} is a simple non-glycosylated cysteine-rich protein, and constitutes one of three predominant proteins found in human seminal fluid along with Prostate Specific Antigen (PSA) and Prostate Acid Phosphatase (PAP). PSP^{94} has a molecular weight of 10.7 kDa, and the complete amino acid sequence of this protein has already been determined (SEQ ID NO:1). The cDNA and gene for PSP^{94} have been cloned and characterized (Ulvsback, et al., Biochem. Biophys. Res. Comm., 164:1310, 1989; Green, et al., Biochem. Biophys. Res. Comm., 167:1184, 1990). Immunochemical and in situ hybridization techniques have shown that PSP^{94} is located predominantly in prostate epithelial cells. It is also present, however, in a variety of other secretory epithelial cells (Weiber, et al., Am. J. Pathol., 137:593, 1990). PSP^{94} has been shown to be expressed in prostate adenocarcinoma cell line, LNCap (Yang, et al., J. Urol., 160:2240, 1998). As well, an inhibitory effect of exogenous PSP^{94} on tumour cell growth has been observed both in vivo and in vitro (Garde, et al., Prostate, 22:225, 1993; Lokeshwar, et al., Cancer Res., 53:4855, 1993), suggesting that PSP^{94} could be a negative regulator for prostate carcinoma growth via interaction with cognate receptors on tumor cells.

Native PSP^{94} has been shown to have a therapeutic modality in treating hormone refractory PCa (and potentially other prostate indications).

Metabolic and immunohistochemical studies have shown that the prostate is a major source of PSP^{94}. PSP^{94} is involved in the feedback control of, and acts to suppress secretion of, circulating follicle stimulating hormone (FSH) both in-vitro and in-vivo in adult male rats. PSP^{94} acts both at the pituitary as well as at the prostate site since both are provided with receptor sites for PSP^{94}.

Both PSA and PAP have been studied as tumour markers in the detection of prostate disease, but since both exhibit elevated levels in prostates having benign prostatic hyperplasia (BPH), neither marker is specific and therefore they are of limited utility.

Recently, it has been shown that PSP^{94} concentrations in serum of patients with BPH or CaP are significantly higher than normal. The highest serum concentration of PSP^{94} observed in normal men is approximately 40 ng/ml, while in men with either BPH or CaP, serum concentrations of PSP^{94} have been observed in the range from 300-400 ng/ml. Because there exists some overlap in the concentrations of PSP^{94} in subjects having normal prostates and patients exhibiting either BPH or CaP, serum levels in and of themselves are of little value.

A major therapy in the treatment of prostate cancer is androgen-ablation. While most patients respond initially to this treatment, its effectiveness decreases over time, possibly because of the presence of a heterogenous population of androgen-dependant and androgen-independent cells to the androgen treatment, while any androgen insensitive cells present would continue to proliferate unabated.

Other forms of cancer which are currently exacting a heavy toll are breast cancer in women and cancer of the gastrointestinal tract. Currently, the use of various cancer drugs such as mitomycin, idarubicin, cisplatin, 5-fluoro-uracil, methotrexate, adriamycin and daunomycin form part of the therapy for treating such cancers. One drawback to such a therapeutic treatment is the presence of adverse side effects due to the drugs in the concentration ranges required for effective treatment.

Accordingly, it would be advantageous to find a more effective means of arresting the growth of prostate, breast and gastrointestinal cancer cells and tumours which can be used effectively against both androgen sensitive and androgen insensitive cells.

In our previous work, described in United States Patent No. 5,428,011, we provided pharmaceutical preparations of native human seminal plasma PSP^{94} for inhibiting in-vitro and in-vivo cancerous prostate, gastrointestinal and breast tumours. The pharmaceutical preparations included native human seminal plasma PSP^{94} which could be administered in an appropriate dosage form, dosage quantity and dosage regimen to a patient suffering from prostate cancer. In another embodiment, the pharmaceutical preparation included a mixture of human seminal plasma PSP^{94} and an anticancer drug which may be administered in an appropriate dosage form, dosage quantity and dosage regimen to a patient suffering from, for example gastrointestinal cancer.

PSP^{94} sourced from human seminal fluid carries with it significant risk of contamination with infectious agents (e.g., HIV, hepatitis (a, b, or c), and other viruses and/or prions). Even with the use of harsh chemical treatment, total eradication of such agents cannot be guaranteed. Additionally, human

seminal fluid is found in limited supply, thus making bulk production of PSP^{94} very difficult. Therefore, the acceptability of human or even xenogeneic sourced PSP^{94} may be very difficult for both the regulatory authorities and the marketplace.

Therefore, the use of recombinant technology for producing PSP^{94} would represent a significant advancement, as recombinant PSP^{94} could be produced both free of pathogens and in an unlimited supply. Furthermore, the material would be homogeneous from a single lot source, avoiding batch to batch variation.

SUMMARY OF THE INVENTION

The present invention relates to the use of recombinant human rHu PSP^{94} (SEQ ID NO: 2) for inhibiting the growth of prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial, ovarian and other cancers of epithelial secretion, or benign prostate hyperplasia (BPH). Preferably, the rHu PSP^{94} is used in a dosage range from about 500 picograms/kg/day to about 1 milligram/kg/day.

The recombinant human rHu PSP^{94} has been expressed in *Pichia pastoris* (host strain GS115 (his4)), is non-glycosylated and has 10 cystein residues. The molecular weight of rHu PSP^{94} was determined as 11.5 kDa, compared to 10.7 kDa for its native counterpart. An MTS cell proliferation assays showed that rHu PSP^{94} could produce a dose dependent decrease in cell numbers of PC-3 human prostate adenocarcinoma cell line.

The invention also relates to the use of at least one peptide selected from the group consisting of the decapeptide depicted in SEQ ID NO: 3, the peptide analogue depicted in SEQ ID NO:4, the peptide analogue depicted in SEQ ID NO:5, and the

peptide analogue depicted in SEQ ID NO:6, or a mixture thereof, for inhibiting growth of prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial, ovarian and other cancers of epithelial secretion, or b nign prostate hyperplasia (BPH). Preferably, the peptide is used in a dosage range from about 250 nanograms/kg/day to about 1 milligram/kg/day.

In another aspect of the invention there is provided a method for treating a patient with prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial, ovarian and other cancers of epithelial secretion, or benign prostate hyperplasia (BPH), comprising administering to the patient a pharmaceutical preparation comprising a peptide selected from the group consisting of rHu PSP^{94} (SEQ ID NO:2), the decapeptide depicted in SEQ ID NO: 3, the peptide analogue depicted in SEQ ID NO: 4, the peptide analogue depicted in SEQ ID NO: 5, and the peptide analogue depicted in SEQ ID NO:6.

The present invention also provides a composition for the use of inhibiting the growth tumours in patients suffering from prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial, ovarian and other cancers of epithelial secretion as well as benign prostate hyperplasia (BPH). The composition comprises recombinant human rHu PSP^{94} (SEQ ID NO: 2), preferably present in a dosage range of about 5 nanograms/kg/day to about 10 micrograms/kg/day. The composition preferably also includes an anticancer drug. A variety of suitable anticancer drugs are known in the art. Examples include, without limitation, mitomycin, idarubicin, cisplatin, 5-fluoro-uracil, methotrexate, adriamycin and daunomycin.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts mass spectrometry analysis of peptide (7-21) (SEQ ID NO: 4).

Figure 2 depicts mass spectrometry analysis of peptide (31-45) (SEQ ID NO:5).

Figure 3 depicts mass spectrometry analysis of peptide (76-94) (SEQ ID NO:6).

Figures 4 - 6 are graphs depicting the inhibitory activity of the decapeptide of SEQ ID NO: 3 on in-vitro PC-3 cells.

Figure 7 depicts a gel showing DNA fragmentation following treatment of PC-3 cells with the peptide depicted in SEQ ID NO:5.

Figure 8 is a graph depicting the results of an apoptosis assay with an ELISA plus kit following peptide treatment of ATCC-PC3 cells for 72 hours.

Figure 9 is a graph depicting growth of fibroblast cells when exposed to PSP^{94} and its analogues in-vitro for 72 hours.

Figure 10 is a graph depicting the effect of the peptides depicted in SEQ ID NOs:4 and 6 on the growth of ATCC-PC3 cells in-vitro at 72 hours.

Figure 11 is a graph depicting the effect of peptides of the invention on growth of ATCC-PC3(OPTI) cells at 72 hours of exposure in-vitro.

Figures 12 and 13 are graphs depicting the results of studies of the anti-tumor efficacy validation of $rPSP^{94}$ against Mat Ly Lu (MLL) tumor implanted in nude mice.

Figure 14 is a graph depicting tumor growth reduction in rHu PSP^{94}-treated mice.

Figures 15 - 17 are graphs depicting tumor growth reduction in decapeptide (SEQ ID NO:3)-treated mice.

DETAILED DESCRIPTION OF THE INVENTION

Various in-vivo and in-vitro experimental studies have been carried out and are summarized herein below to determine the efficacy of concentrations of rHu PSP^{94} (SEQ ID NO: 2) higher than native PSP^{94} concentrations secreted by the diseased prostate as tumour suppressive agent for arresting or inhibiting the growth of prostatic adenocarcinoma. Studies have also been carried out to determine the efficacy of decapeptide (SEQ ID NO:3) and synthetic peptide analogues of rHu PSP^{94} (SEQ ID NO: 4, No. 5 and No.6) as tumour suppressive agents.

Studies were carried out using PC-3 human prostate adenocarcinoma line which can be maintained both in vivo as a xenograft in nude mice and in vitro as a cell line. In addition, a rat Dunning Mat LyLu prostate tumour, which is a pre-eminent animal model for the study of CaP, was also used. The Dunning tumour is a fast growing, poorly differentiated, transplantable tumour which can be maintained both in-vivo in the Copenhagen rat and in-vitro as a cell line.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

PREPARATION OF rHu PSP^{94} (SEQ ID NO: 2)

rHu PSP^{94} was cloned and expressed in *Pichia pastoris*, and then purified and characterized as follows.

Materials

DEAE-cellulose (DE52) was from Whatman, Fairfield, NJ and the dialysis tubing from BioLynx (Pierce Inc.). Broad-range molecular weight markers and Econo-pack columns fitted with flow adapters were from Bio-Rad Labs Ltd CA. Pellicon device was from Millipore, MA. Tris-HCl was obtained from ICN, and MES from Sigma. Other reagents were obtained from standard commercial sources and were of reagent grade. ECL detection kit was purchased from Biolynx Canada and swine anti-rabbit IgG alkaline-phosphatase conjugates from DAKO, Denmark. Rabbit polyclonal antiserum against PSP94 was a gift from the late Dr. A. Sheth. *Pichia* expression Kit version G was from Invitrogen, Carlsbad, CA and the Non-Radioactive High Prime DIG labeling kit® was from Boehringer Mannheim Indianapolis, IN. The MTS assays were performed using Cell Titer Aqueous Non radioactive cell proliferation assay kit from Promega Madison, WI. MRX microtiter plate reader was from Dynex technologies, Chantillly, VA. All primers were synthesized by Procyon Biopharma Inc. London, ON.

Cell lines and Cell cultures

P. pastoris host strain GS115 (*his4*) and all the Pichia related products were obtained from Invitrogen. PC3 (ATCC-# CRL 1435) cell line was from the American Type Cell Culture (ATCC) and maintained in OPTI MEM media with10 % FBS. All cell culture products were obtained from GIBCO BRL.

Cloning and Expression

TA cloning vector (pCR ™ 2.1) containing human PSP94 cDNA with 20 a.a. leader sequence used previously (9)(Baijal et. al. 1999) was modified without its leader sequence to amplify human PSP94. The primers for the PCR reaction were designed to contain EcoRI restriction sites at either end. The 5′ primer used was 5′-GGG AAG ↓AAT TCT CAT GCT ATT TCA TA-3′ and the 3′ primer, 5′-TGG ATA TCT GCA GAA TT↓C GGC-3′. The EcoRI cleavage signal has been designated with ↓ and the +1 start site for PSP94 (Ser), has been underlined. The subcloning of the PSP94 insert was done in pPIC9 vector (Invitrogen), which contained EcoRI restriction site at the 5′ end, about 5-8 amino acids away from the cleavage site that removes the signal sequence of the PSP94 insert. The amplification of the product was done by PCR, using BM Expand™ High Fidelity PCR System.

The PCR included 1 cycle for 12′ @ 94 C, 25 cycles for 1′ @ 94 C followed by 1′ @ 55 C, 1′ @ 72 C and final 10′ @ 72 C. The product was run on 1.5 % agarore gel and appropriate bands isolated using Pharmacia Sehphaglass Kit (Bandprep). The EcoRI enzyme was used for the restriction digestions of both the plasmid and the PCR products followed by ligation and transformations, using DH5α cells. The isolated clones were selected for by ampicillin resistance and inserts were identified by restriction mappings. The constructs were sequenced (Robart's sequencing service, London ON) to identify PSP94 insert with correct orientation and reading frame as well scrutinized for point mutations.

To follow the time course of expression and to determine the optimum time for harvesting secreted PSP94, daily aliquots of the expression medium were removed beginning with the first day of induction, until the time of harvesting (96 h). Expression of the protein was tracked by SDS PAGE with 15 % gel,

followed by Western blot analysis using polyclonal antibody raised against native PSP94.

Screening for High Expressing Clones

About 100 clones were picked and grown into 2 ml culture media to OD_{600} of ~6. Total DNA was isolated for rapid dot blot technique to detect multiple integration by Southern blot analysis to screen for high PSP94 expressing clones. 200ul of each culture specimens were denatured and blotted to a positively charged nylon membrane, placed in the dot blot apparatus (in duplicate) and air-dried. The membrane was soaked between two sheets of Whatman 3MM paper for 15 min in a solution with 50 mM EDTA, 2.5 % BME, pH 9, followed by 24 hours at 37 C with 1 mg/ml Zymolyase 100T, 5min in 0.1 N NaOH, 1.5 M NaCl, 0.015M sodium citrate pH 7 and 2 x 5 min in 2xSSC. Finally the membrane was baked at 80 C for 45 min and UV exposed for 15min. Human PSP94 cDNA probe was labeled with the non radioactive High Prime DIG labeling kit® (Boehringer Mannheim) and was used for hybridization (9). All DIG labeling procedures were performed according to the manufacturer's protocol and the hybridization signals were detected on the Hyper film-ECL (Amersham Life Science Inc. Arlington Hts, IL).

Hybridization with DIG labeled cDNA probe (25ng/ul) was done for 2 days at 42 C in high SDS buffer (SDS 7 %; formamide, 50%; 5 X SSC; 50 mM sodium phosphate, pH 7.0; N-lauroyl-sarcosine, 0.1 % (w/v)) and blocking reagent, 2 %. CSPD® (Boehringer Mannheim) was used as the chemiluminescence substrate. The clone with highest signal intensity was used for all flasks shaken cultures.

Optimization of the Expression of the Protein in the Flask Shaken Cultures

A single clone containing the PSP94 construct was selected for the optimum expression of the protein. 25ml of BMG medium was grown until the OD_{600} measured between 2 and 6. The flask shaken cultures in I liter volumes of BMG media were inoculated with t25ml culture and incubated in Baffled Erlenmeyer flasks for growth phase, until OD_{600} reached approximately 2.0 to 6.0. The culture was centrifuged for 15min at 2500 X g and the pellet collected. The growth in the induction phase was then carried out in BMM media by inoculating it with the collected cell pellet in Baffled flasks and grown for 6 days, as described by Invitrogen. The volume of BMM varied based on the amount of pellet. Five ml of 100% methanol was added per litre of culture, each day, around the same time, to a final concentration to 0.1 %. The plasmid without the insert served as a negative control.

To determine the optimum time for harvesting secreted PSP94, aliquots were monitored every 24 hrs for 6 days, beginning with the first day of induction. The levels of PSP94 protein were determined by measuring the cell densities at OD_{600} and by15 % Comassie stained SDS PAGE and Western blot analysis, using polyclonal antibody against native PSP94.

Sample Preparation

The specimen from the flask shaken culture with the highest rPSP94 expression, post induction growth (96 hr), was centrifuged at 2500 X g for 20min. The supernatant was filtered through 0.8µm filter and concentrated approximately 10-fold using the Pellicon unit (Millipore). The specimen was dialyzed against 0.05 mM Tris HCl buffer, pH 8.0, using a M_r 3500 cut-off tubing. The final supernatant was tested by SDS PAGE and Western blot analysis and further used for purification.

Culture Conditions for Fermentation

The fermentation was carried out at the Institute for Biological Sciences, National Research Council Ottawa ON Canada, following the manufacturer's protocol. The fermentation procedure was initiated by inoculating 7.5-liter medium with 625 ml of starter flask shaken culture material. The growth phase culture was carried out for 2 days in BMG medium until the OD_{600} reached approx. 0.5. The initial growth phase was terminated and induction initiated by the addition of 100 % methanol, as described by inVitrogen. The culture was finally harvested at 95 hr after being on methanol for 67 hrs. The final volume of the culture was approximately 13.5 lit.

Sample Preparation using Fermentation Culture

The large cell mass was removed by centrifugation and was performed at NRC. The cell free medium collected (9 lit) was further clarified by 0.2 u filtration Pellicon unit. The final 8.5 lit containing secreted rPSP94 was tested for rPSP94 expression and saved at -20 C for isolation and purification of the protein.

Protein Estimation

The protein in the culture supernatants of the flask shaken and the fermentation was based on band intensities of various amounts of the supernatant samples on the SDS PAGE gels. Standard curve were constructed by electrophoresis of increasing predetermined quantities of pure lyophilized PSP94 on the same gel. The initial estimate for rPSP94 at each step of purification was determined by OD_{280}. Quantification of protein at the final steps of purification was done by BCA method, using bovine serum albumin as standard.

Lyophilization

Samples of purified rPSP94 were dialyzed against deionized water using M_r 3000 cut-off membrane and lyophilized.

SDS gel electrophoresis

SDS gel electrophoresis was performed using 15% acrylamide separating and 5% stacking gels, containing 0.1% SDS according to Laemmli under reducing conditions. Broad-range molecular weight markers were used for the estimation of molecular weights of the protein. Protein bands were stained with Coomassie Brilliant Blue R-250.

Western Blotting

For immunoblotting, Mini Trans-Blot Electrophoretic Transfer Cell (Bio Rad) was used with Hi bond-C supper membrane (Amersham) and 85mm blotting papers. Protein samples, 0.4 µg each, run on SDS PAGE gel, as described earlier, were transferred to the membrane using transfer buffer (25 mM Tris, 192 mM Glycine, pH 8.3 and 20 % methanol) at 200 mA° for 2hrs at 4° C. Membranes were blocked O/N by incubation in 2% (w/v) non-fat dry milk (skim milk) dissolved in 500 mM NaCl, 20 mM Tris-HCl, pH 7.5 (TBS) at room temp (RT). Immunoblots were washed three-times with TBS containing 0.02% (v/v) Tween 20 (TTBS). The blot was subsequently incubated with anti PSP94 (1:2000 dilution) in TTBS with 2% skim milk for 2 hrs at RT. Membranes were washed thrice with TTBS, 5min each, incubated with secondary antibody, swine anti rabbit antibody HRP conjugated ((1:5000) in TTBS at RT and washed thrice with 0.02% TTBS 5 minutes each. Blots were developed using the ECL detection system, as per manufacturer's instructions, using the Super Signal Substrate, and exposed to a Hyperfim ECL from Amersham LS for 5 to 20 sec. Pre-stained molecular weight markers were used for the estimation of molecular weights of the protein.

DE52 Column Chromatography

After removal of P. *pastoris* cells from the fermentation culture, PSP94 containing supernatant was concentrated ~ ten fold, dialyzed and subjected to anion exchange chromatography. A column of DE52 with a bed volume ~ 40ml (2.5 (id) X 8 cm (h) cm) was equilibrated with 0.05 M Tris-HCl, pH 8.0. The sample (25 ml) containing 15-20 mg PSP94 protein was applied to the DE52 column at a flow rate of 1 ml/min. Impurities from the column were removed by washing it with 40-50 ml of the equilibrating buffer, and monitoring the A_{280}. This step was followed by 100-150 ml of 0.05 M Tris-HCl, pH 6.5 and most importantly till the pH of the wash was ~6.5. The column was further washed with 100- 150 ml of 0.05 M MES-acetate buffer, pH 6.5, until the A_{280} value approached zero. Finally rPSP94 was eluted from the column with 0.05 M MES-acetate buffer, pH 5.0. Peak fractions were identified by A_{280} determination, followed by SDS PAGE and Western blot analysis as described above. Fractions with high A_{280} values (0.5 to 1.8) were pooled and dialyzed against water/PBS for storage at -20 C and/or lyophilization.

Amino acid Composition

The amino acid analysis of the DE52 purified flask shaken and the fermentation specimens was carried out at the Louisiana State University Medical Core Laboratory. The Perkin Elmer Biosystems Derivatizer-Analysis system was used with Spheri-5 PTC C-18 5u column and UV detection at OD_{254}.

Mass Spectral analysis

Purified samples from the flask shaken and the fermentation cultures were analyzed using the PerSeptive Biosystems (Framingham, MA), with Voyager-DE MALDI-TOF mass spectrometer using 337 nm light from a nitrogen laser at the Louisiana State University Medical Core Laboratory. About 50

scans were averaged for each analysis. A sample from the native PSP94 was also analyzed under similar conditions for comparison.

EXAMPLE 2

EFFECT OF rHu PSP^{94} ON IN-VITRO PC-3 CELLS (MTS ASSAY)

The biological activity of the rHu PSP^{94} was determined by its growth inhibitory effects on the human prostate cancer cells PC-3. The cell proliferation was monitored on PC-3 cells using the MTS/PMS kit (Promega), which primarily measures the mitochondrial activity of live cells. In addition, a visual observation of the cells was also done to check the cell morphology, which could also result in inhibition of cell growth. These results are summarized in Table 1. The following conditions for MTS assay were used:

PC-3 (ATCC, Lot AT06), Passage Number $n \geq 70$, Cell line adapted to grow in serum-free OPTI-MEM. Continuous exposure for 72 hrs without changing medium (adding PSP^{94} at 2X concentration directly to wells and diluting it 1:2 to 1X to minimize cell manipulation and avoid detachment). Evaluation of rHu PSP^{94} demonstrated a substantial reduction in cell numbers ranging from 37-57% at 80 and 120 µg/ml concentrations, respectively in 3 out of 4 experiments (Table 1). Results of Trypan Blue Exclusion Test demonstrated 62% inhibition of cell viability at 80 µg/ml.

TABLE 1: Effect of rHu PSP^{94} on in vitro PC-3 cells (MTS assay)

Reference:
(Monthly Activity Report June 2000)

Experiment #	rHu PSP^{94}	% Viability (control = 100%) at µg/ml			
		40	60	80	120
079-140	00-133F	72	78	58	43
079-105	"	63	63	63	68
079-120	"	95	85	78	ND
079-153	"	100	52	62	60
079-140	99.09.08	100	98	90	52

rHu PSP^{94}	% Viability (control = 100%) at µg/ml				
	5	10	20	40	80
00-133F	98	84	78	70	55
"	92	95	80	71	59
"	89	69	79	68	65

EXAMPLE 3

EFFECT OF rHu PSP^{94} ON IN-VITRO PC-3 CELLS ($[^3H]$-THYMIDINE UPTAKE ASSAY)

The vitro rHu PSP^{94} efficacy validation was assessed in the $[^3H]$-Thymidine Uptake assay where PC-3 cells are exposed to various concentrations of rHu PSP^{94} for 72 hours and during the final 16 hrs of incubation the cells are pulsed with 1 µCu of $[^3H]$-Thymidine. These results are summarized in Table 2. Evaluation of rHu PSP^{94} demonstrated a 65% reduction in the %-age of cells incorporating $[^3H]$-thymidine following treatment at 80 µg/ml for 72hrs. For comparison of sensitivities between $[^3H]$-Thymidine Uptake and the MTS assays an additional plate was set aside for MTS assay evaluation and treated in parallel with the same lot of rHu PSP^{94}. The results demonstrated a 35% reduction in cell viability (65% cells remaining viable) following treatment at 80 µg/ml, indicating that the $[^3H]$-Thymidine Uptake assay might be more sensitive than the MTS assay.

TABLE 2: Effect of rHu PSP94 on in vitro PC-3 cells ([3^H]-Thymidine uptake assay)

Experiment #	PSP^{94}	[3^H]-Thymidine Uptake (% of control) at µg/ml				
		5	10	20	40	80
1	rHu PSP^{94} (00-133F)	94	101	98	79	35
1	n PSP^{94} 99413S (B2)	97	98	100	98	77

EXAMPLE 4

EFFECT OF DECAPEPTIDE AND OTHER PEPTIDE ANALOGUES ON IN-VITRO PC-3 CELLS

The synthetic decapeptide (SEQ ID NO: 3) has been shown herein to mimic the biological action of native nPSP^{94} (SEQ ID NO: 1) and therefore its effect on the PC-3 cells was studied in clonogenicity assay. Referring to Figures 4-6, the decapeptide (SEQ ID NO: 3) has a similar inhibitory action as nPSP^{94} (SEQ ID NO: 1) on in-vitro PC-3 cells was studied. Specifically, a 40% colony count inhibition was observed with 1 ug/ml of the decapeptide (SEQ ID NO: 2) leading to a maximum of 60% inhibition at 10 µg/ml.

EXAMPLE 5

DNA FRAGMENTATION ASSAY

DNA fragmentation assay measures the extent of apoptosis by the presence of DNA ladder on 1.2% Agarose gel following exposure of PC-3 to various concentrations of the peptide analogues. Peptide analogues used: peptide 7-21 (SEQ ID NO: 4), peptide 31-45 (SEQ ID NO:5) and peptide 76-94 (SEQ ID NO:6). Duration of treatment 72 hrs. DNA was isolated and run

on 1.2% Agarose gel. Results demonstrated that all the peptides tested produced a DNA laddering effect characteristic of apoptosis. This effect was especially evident following treatment with peptide 31-45 (SEQ ID NO: 5) (Figure 7).

EXAMPLE 6

APOPTOSIS ASSAY BY ELISA PLUS

The three peptides analogues (SEQ ID NO: 4, No.5 and No. 6) and native PSP^{94} as a positive control were tested in ELISA plus assay to measure cell death through apoptosis. The PC-3 cells were treated with various concentrations of peptides for 72 hrs. It was observed that a dose dependent increase in the apoptotic cell death was noticed in all the 3 peptides analogues (SEQ ID NO: 4, No.5 and No. 6). The middle portion peptide (SEQ ID NO: 5) was more potent at 90 µm concentration (Figure 8). Apoptosis assay was done as per instructions in the ApopTag kit (Boerenger Mannheim).

EXAMPLE 7

INHIBITION OF CELL-GROWTH BY PSP^{94} PEPTIDE ANALOGUES

The biological activity of the peptide analogues (SEQ ID NO: 4, 5 and 6) was determined by their growth inhibitory effects on the human prostate cancer cells PC-3. The cell proliferation was monitored on PC-3 cells and control normal fibroblast using the MTS/PMS kit (Promega), which primarily measures the mitochondrial activity of live cells. In addition, a visual observation of the cells was also done to check the cell morphology, which could also result in inhibition of cell growth. These results are show in Figures 9 and 10. No cell inhibitory effect was observed against fibroblast cells following incubation with various peptide concentrations for 72

hrs (Figure 9 and Figure 11) whereas significant growth inhibition was observed especially with middle portion peptide fragment 31-45 (SEQ ID NO: 5) as well as the amino and carboxy terminus peptide fragments (SEQ ID NOs: 4 and 6, respectively) (Figure 10).

EXAMPLES 8 & 9

IN-VIVO EXPERIMENTS (Figures 12 & 13)

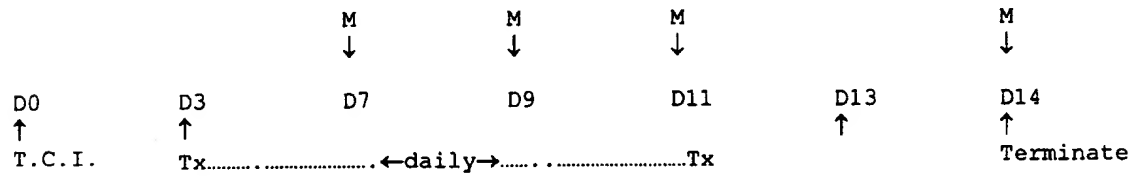
(Study MLL-1)

Anti-tumor Efficacy Validation of $rPSP^{94}$ against Mat Ly Lu (MLL) Tumor Implanted in **Nude Mice.**
(Protocol based on S. Garde et al.; The Prostate, 22: 225-233, 1993 "Effect of Prostatic Inhibin Peptide (PIP) on Prostate Cancer Cell Growth In Vitro and In Vivo")

Groups (n = 8 mice / group):

- **Group 1**: Negative control PBS s.c.
- **Group 2**: Positive control Doxorubicin @ 5mg/kg i.v. single bolus on Day 3
- **Group 3**: $rPSP^{94}$ Batch # 00-133F @ 1µg/kg s.c.
- **Group 4**: $rPSP^{94}$ Batch # 00-133F @ 10µg/kg s.c.
- **Group 5**: $rPSP^{94}$ Batch # 00-133F @ 100µg/kg s.c.

Schematic:



(Study MLL-2)

Anti-tumor Efficacy Validation of $rPSP^{94}$ against Mat Ly Lu (MLL) Tumor Implanted in **SCID Mice.**
(Protocol based on S. Garde et al.; The Prostate, 22: 225-233, 1993 "Effect of Prostatic Inhibin Peptide (PIP) on Prostate Cancer Cell Growth In Vitro and In Vivo")

Groups (n = 8 mice / group):

- **Group 1**: Negative control PBS s.c.

- **Group 2:** Positive control Doxorubicin @ 5mg/kg i.v. single bolus on Day 3
- **Group 3:** rPSP^{94} Batch # 00-133F @ 1µg/kg s.c.
- **Group 4:** rPSP^{94} Batch # 00-133F @ 10µg/kg s.c.
- **Group 5:** rPSP^{94} Batch # 00-133F @ 100µg/kg s.c.

Schematic:

		M ↓	M ↓	M ↓		M ↓
D0 ↑	D3 ↑	D7	D9	D11	D13 ↑	D14 ↑
T.C.I.	Tx.....←daily→.....Tx					Terminate

Conclusions For Examples 8 & 9:

- Difference in tumor size and growth in Nude vs SCID. The tumors were smaller in SCID mice. Maybe due to some specific factors controlling tumor growth.
- Good tumor reduction with Doxorubicin (good positive control):
  - Tumor Weight Reduction in Nude mice (MLL-1): **48% p=0.006**
  - Tumor Weight Reduction in SCID mice (MLL-2): **82% p=0.002**
- Significant tumor reduction with rPSP^{94} @ 1µg/kg:
  - Tumor Weight Reduction in Nude mice (MLL-1): **26% p=0.042**
  - Tumor Weight Reduction in SCID mice (MLL-2): **65% p=0.010**

EXAMPLE 10 (Figure 14)

IN-VIVO EXPERIMENT USING PC-3 CELL LINE

PC3 human prostate tumor (ATCC 1435) obtained from ATCC was implanted into nude mice and tumor growth monitored for approximately 18 days. The PC-3 cells were injected once subcutaneously into the shoulder. Once tumor growth has been established (50 mm^3 volume) the treatment with rHu PSP^{94} (SEQ ID

NO: 2) begun at q1dx14 s.c. based on the following assigned treatment groups (n= 8 per group):

Treatment Groups	Test and control Articles	Dose Level* (µg/kg)	Dos Conc. (µg/mg)	No of Animal
1 (Neg Control)	PBS	0	0	8
2 (Pos Control)	Doxorubicin	5000**	2500	8
3	rHu PSP^{94}	1	0.5	8
4	rHu PSP^{94}	10	5	8
5	rHu PSP^{94}	100	50	8
6	rHu PSP^{94}	1000	500	8

Results of this experiment (Figure 14) demonstrated tumor growth reduction in the rHu PSP^{94} treated group of mice at a dosage level of 1 µg/kg body weight per day similar to that of Doxorubicin (given at 5 mg/kg) a chemotherapeutic agent used as reference gold standard.

EXAMPLE 11 (Figures 15-17)

IN-VIVO EXPERIMENT USING PC-3 CELL LINE

PC3 human prostate tumor (ATCC 1435) obtained from ATCC was implanted bilateraly into nude mice and tumor growth monitored for approximately 18 days. The PC-3 cells were injected once subcutaneously into each flank. Once tumor growth has been established (0.25-0.50 cc volume) the treatment with decapeptide PB102 (SEQ ID NO:3), native PSP^{94} (PB100) (Sequence ID. 1) and control irrelevant decapepride PB111

begun at q1dx14 s.c. based on the following randomly assigned treatment groups (n=4 per group):

Treatment Groups	Test and control Articles	Dose Level* (µg/kg)	Dose Conc. (µg/mg)	No of Animal
1 (Neg Control)	PBS	0	0	4
3	PB102 (decapeptide)	1	0.5	4
4	PB102 (decapeptide)	10	5	4
5	PB102 (decapeptide)	100	50	4
6	PB102 (decapeptide)	1000	500	4
7	PB100 ($nPSP^{94}$)	1	0.5	4
8	PB100 ($nPSP^{94}$)	10	5	4
10	PB100 ($nPSP^{94}$)	100	50	4
11	PB100 ($nPSP^{94}$)	1000	500	4
12	PB111 (control)	1	0.5	4
13	PB111 (control)	10	5	4
14	PB111 (control)	100	50	4
15	PB111 (control)	1000	500	4

Results of this study (Figures 15-17) demonstrated significant ($p<0.05$) tumor growth reduction in the decapeptide PB102- treated group of mice at a dosage level of 10 µg/kg body weight per day.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. The citation of any publication is for its disclosure prior to the

filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

SEQ ID No: 1
(nPSP94 sequence):

NH
SCYFIPNEGVPGDSTRKCMDLKGNKHPINSEWQTDNCETCTCYETEISCCTLVSTPVGYDKDNC
QRIFKKEDCKYIVVEKKDPKKTCSVSEWII COOH

SEQ ID NO: 2
(rPSP sequence):

NH
EAEAYVEFSCYFIPNEGVPGDSTRKCMDLKGNKHPINSEWQTDNCETCTCYETEISCCTLVSTP
VGYDKDNCQRIFKKEDCKYIVVEKKDPKKTCSVSEWII COOH

SEQ ID NO: 3
(decapeptide)

Tyr-Thr-Cys-Ser-Val-Ser-Glu-Pro-Gly-Ile

SEQ ID NO: 4
(N-terminus peptide 7-21)

NH
Asn-Glu-Gly-Val-Pro-Gly-Asp-Ser-Thr-Arg-Lys-Cys-Met-Asp-Leu

SEQ ID NO: 5
(middle portion peptide 31-45)

Glu-Trp-Gln-Thr-Asp-Asn-Cys-Glu-Thr-Cys-Thr-Cys-Tyr-Glu-Thr

SEQ ID NO: 6
(carboxy-terminus 76-94)

Ile-Val-Val-Glu-Lys-Lys-Asp-Pro-Lys-Lys-Thr-Cys-Ser-Val-Ser-Glu-
Trp-Ile-Ile COOH

CLAIMS:

1. Use of human rHu PSP^{94} (SEQ ID NO: 2) for inhibiting growth of prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial, ovarian or other cancers of epithelial secretion, or benign prostate hyperplasia (BPH), wherein the human rHu PSP^{94} is used in a dosage range from about 500 picograms/kg/day to about 1 milligrams/kg/day.

2. The use of human rHu PSP^{94} (SEQ ID NO: 2) according to claim 1 wherein the human rHu PSP^{94} is used in a dosage range from about 5 nanograms/kg/day to about 10 micrograms/kg/day.

3. The use of human rHu PSP^{94} (SEQ ID NO: 2) according to claim 1 wherein the human rHu PSP^{94} is used in a mixture comprising the human rHu PSP^{94} (SEQ ID NO: 2) and a pharmaceutically acceptable carrier.

4. The use of human rHu PSP^{94} (SEQ ID NO: 2) according to claim 3 wherein the human rHu PSP^{94} is used in a dosage range from about 5 nanograms/kg/day to about 500 nanograms/kg/day.

5. The use of human rHu PSP^{94} according to claim 3 wherein the pharmaceutically acceptable carrier includes time release encapsulation means for effecting continual dosing of the human rHu PSP^{94}.

6. The use of human rHu PSP^{94} according to claim 5 wherein the time release means comprises a liposome.

7. The use of human rHu PSP^{94} according to claim 5 wherein the time release means comprises a polysaccharide.

8. The use of human rHu PSP^{94} according to claim 1 wherein human rHu PSP^{94} is used in a mixture including an anticancer drug.

9. The use of humans rHu PSP^{94} according to claim 8 wherein the anticancer drug is selected from the group consisting of mitomycin, idarubicin, cisplatin, 5-fluoro-uracil, methotrexate, adriamycin and daunomycin.

10. Use of at least one peptide selected from the group consisting of the synthetic decapeptide depicted in SEQ ID NO:3, the peptide analogue depicted in SEQ ID NO:4, the peptide analogue depicted in SEQ ID NO:5, and the peptide analogue depicted in SEQ ID NO:6, for inhibiting growth of prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial, ovarian or other cancers of epithelial secretion, or benign prostate hyperplasia (BPH), wherein the peptide is used in a dosage range from about 250 nanograms/kg/day to about 1 milligrams/kg/day.

11. A method for treating a patient with prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial, ovarian or other cancers of epithelial secretion, or benign prostate hyperplasia (BPH), the method comprising administering to the patient a pharmaceutical preparation comprising at least one peptide selected from the group consisting of human rHu PSP^{94} (SEQ ID NO:2), the decapeptide depicted in SEQ ID NO:3, the peptide analogue depicted in SEQ ID NO:4, the peptide analogue depicted in SEQ ID NO:5, and the peptide analogue depicted in SEQ ID NO:6).

12. The method of claim 11 wherein the pharmaceutical preparation includes an anticancer drug.

13. The method of claim 12 wherein the anticancer drug is selected from the group consisting of mitomycin, idarubicin, cisplatin, 5-fluoro-uracil, methotrexate, adriamycin and daunomycin.

14. The method according to claim 13 wherein the peptide is human rHu PSP^{94} (SEQ ID NO: 2) and wherein it is administered in a dosage range from about 25 picograms/kg/day to about 1 milligrams/kg/day.

15. The method according to claim 13 wherein the human rHu PSP^{94} is administered in a mixture including an anticancer drug, wherein the anticancer drug is selected from the group consisting of mitomycin, idarubicin, cisplatin, 5-fluoro-uracil, methotrexate, adriamycin and daunomycin.

16. The method according to claim 14 wherein the human rHu PSP^{94} is administered in a dosage range from about 5 nanograms/kg/day to about 10 micrograms/kg/day.

17. The method according to claim 16 wherein the human rHu PSP^{94} is administered in a mixture including an anticancer drug selected from the group consisting of mitomycin, idarubicin, cisplatin, 5-fluoro-uracil, methotrexate, adriamycin and daunomycin.

18. The method according to claim 16 wherein the human rHu PSP^{94} (SEQ ID NO: 2) is used in a mixture including a pharmaceutically acceptable carrier.

19. The method according to claim 18 wherein the pharmaceutically acceptable carrier includes time release encapsulation means for effecting continual dosing of the human rHu PSP^{94}.

20. The method according to claim 19 wherein the time release encapsulation means comprises a liposome.

21. The method according to claim 19 wherein the time release encapsulation means comprises a polysaccharide.

22. The method according to claim 11 wherein the peptide is administered in a dosage range of about 25 nanograms/kg/day to about 50 micrograms/kg/day.

23. The method according to claim 22 wherein the pharmaceutically acceptable carrier includes time release encapsulation means for effecting continual dosing of the peptide.

24. A composition for inhibiting the growth tumours in patients suffering from prostatic adenocarcinoma, stomach cancer, breast cancer, endometrial, ovarian or other cancers of epithelial secretion, or benign prostate hyperplasia (BPH), comprising:

a) Human rHu PSP^{94} (SEQ ID NO: 2) present in a dosage range of about 5 nanograms/kg/day to about 10 micrograms/kg/day; and

b) an anticancer drug.

25. A composition according to claim 24 wherein said anticancer drug is selected from the group consisting of mitomycin, idarubicin, cisplatin, 5-fluoro-uracil, methotrexate, adriamycin and daunomycin.

26. A composition according to claim 24 including a pharmaceutically acceptable carrier.

27. A composition according to claim 24 including a pharmaceutically acceptable carrier includes time release encapsulation means for effecting continual dosing of the composition.

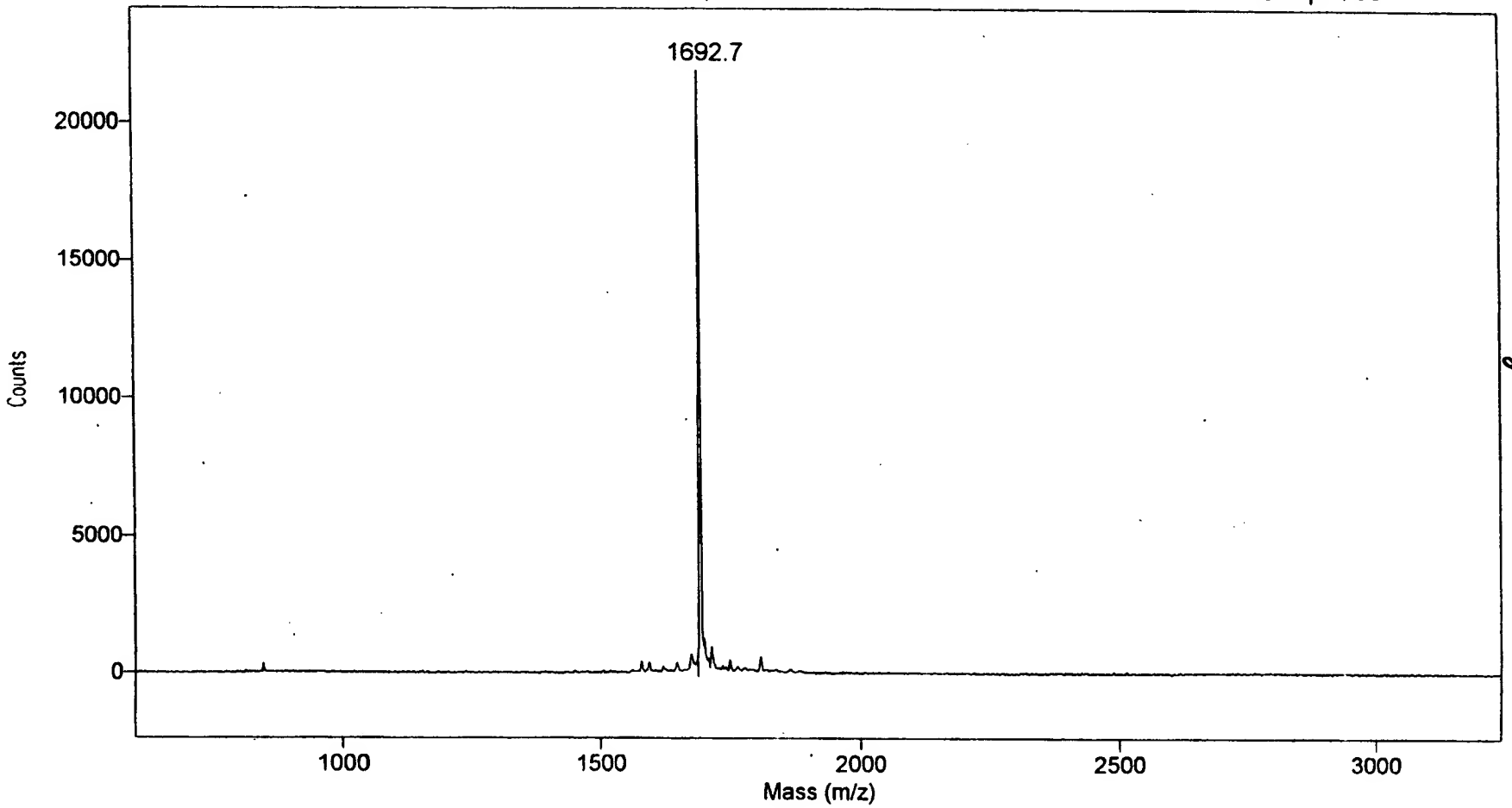
28. A method for treating patients with diseases characterized by elevated levels of FSH comprising administering a pharmaceutical preparation in an appropriate dosage form, the pharmaceutical preparation comprising at least one peptide selected from the group consisting of human recombinant PSP^{94} (SEQ ID NO: 2), the decapeptide depicted in SEQ ID NO: 3, the peptide depicted in SEQ ID NO:4, the peptide depicted in SEQ ID NO:5, and the peptide depicted in SEQ ID NO:6.

Procyon (SEQ ID NO:4)

Sample: PSPa(7-21)
MW= 1691.5

File # 1 : C:\VOYAGER\PROCYON\SMOOTH.MS Collected: 5/4/99 2:10 PM

Sampl : 98



Comment:

Method: LDE1008A	Accelerating Voltage: 20000	Laser : 1860	Low Mass Gate: OFF	Mirror Ratio:
Mode: Linear	Grid Voltage: 94.000 %	Scans Averaged: 12	Timed Ion Selector:	PSD Mirror Ratio:
	Guide Wire Voltage: 0.030 %	Pressure: 7.15e-07		
	Negative Ions: OFF			

41/1

Fig. 1

CA 02321256 2000-10-16

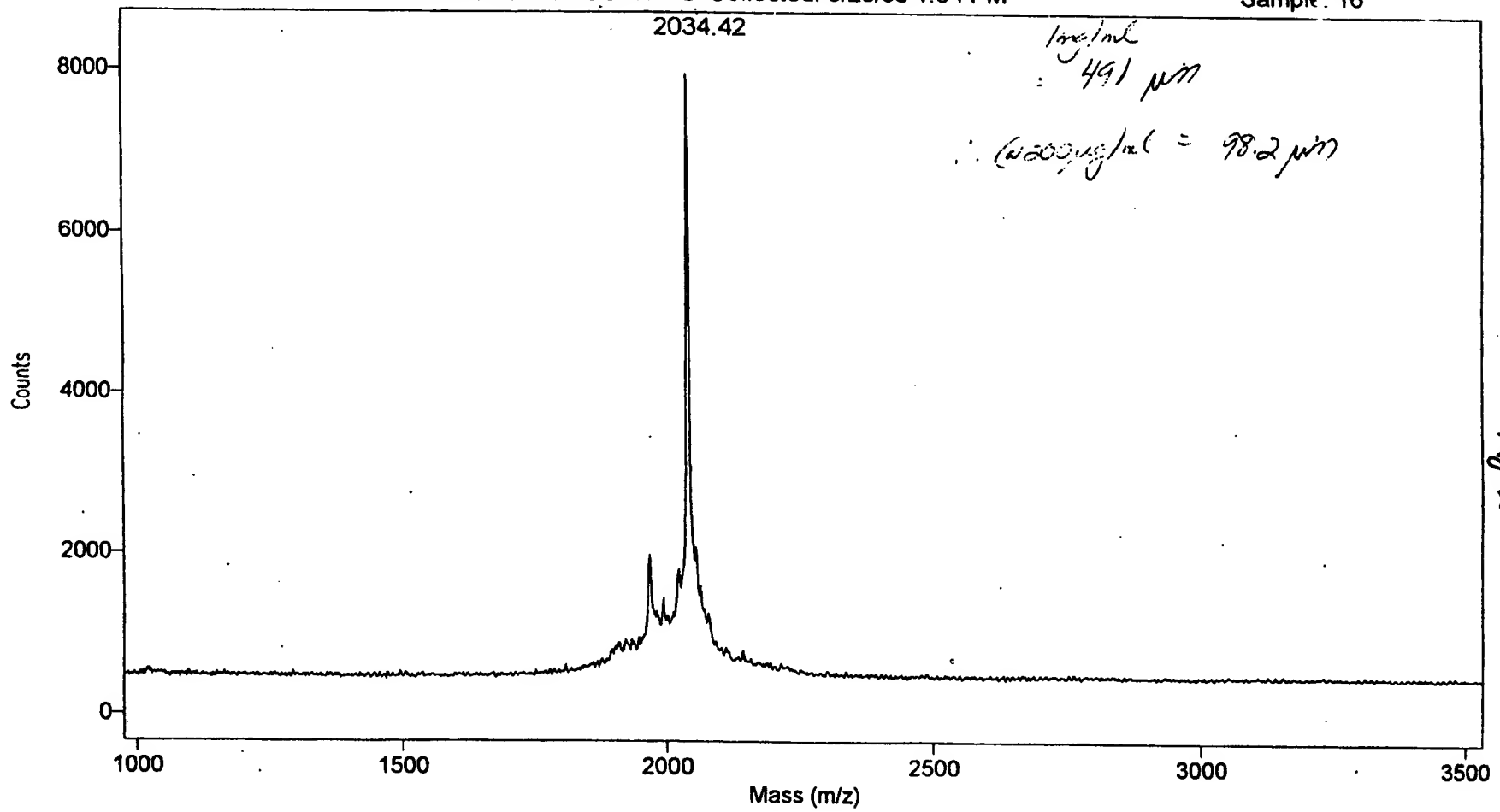
Procyon (SEQ ID NO: 5)

Sample: PSI 'a(31-45)

MW= 2034 9

File # 2 : C:\VOYAGER\PROCYON\SMOOTH.MS Collected: 9/23/98 1:54 PM

Sample : 16



Comment:

Method: LDE1008B
Mode: Linear

Accelerating Voltage: 20000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.060 %
Negative Ions: ON

Laser : 1790
Scans Averaged: 18
Pressure: 2.39e-07

Low Mass Gate: OFF
Timed Ion Selector:

Mirror Ratio:
PSD Mirror Ratio:

CA 02321256 2000-10-16

Fig. 2

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Procyon (SEQ ID NO: 5)

W T

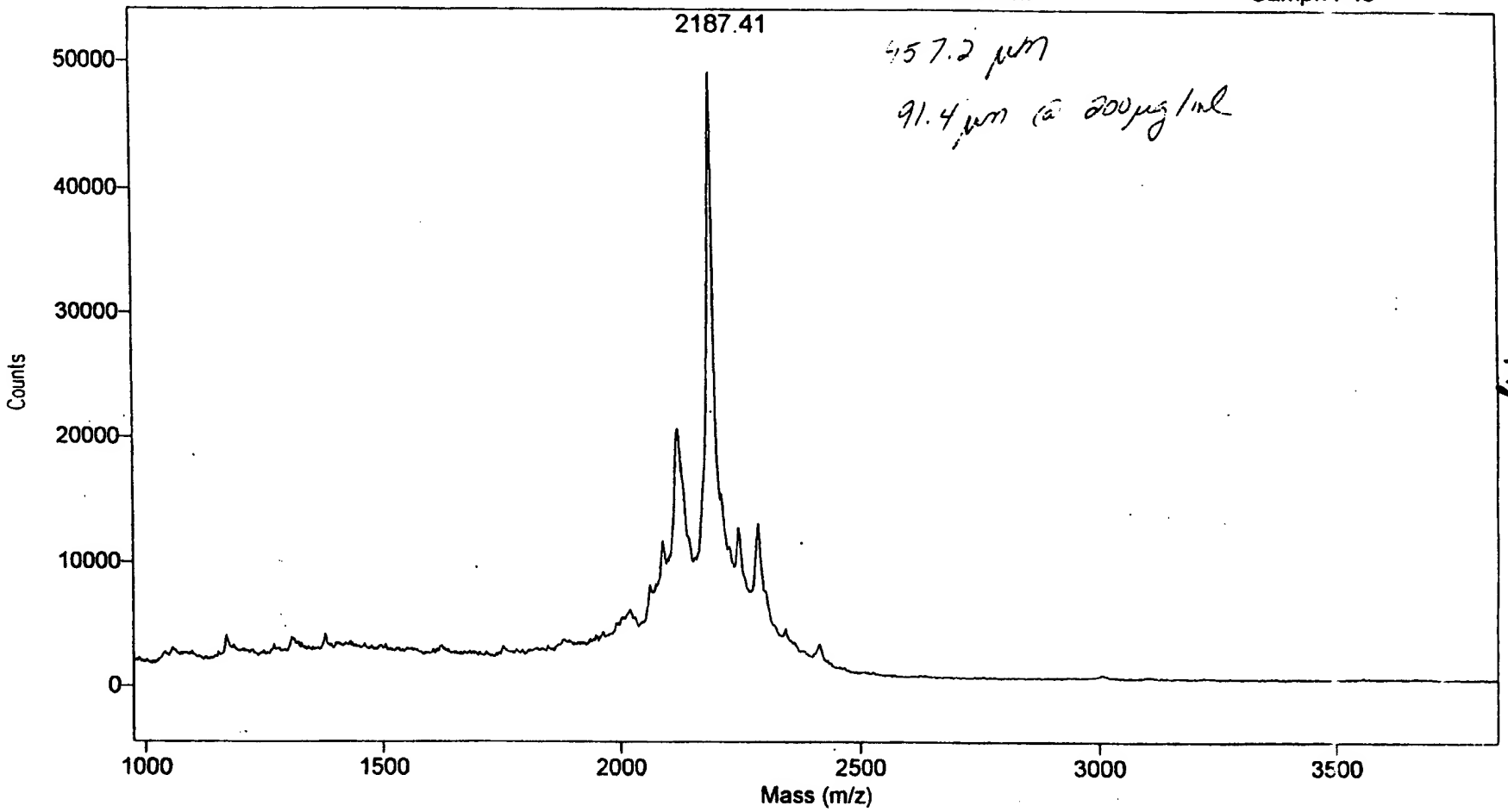
N.B. Thio substitution "Trp à Tyr" (2nd last amino acid by m.state)

Sample: PSMa(76-94)

MW= 2187.2

File # 1 : C:\VOYAGER\PROCYON\SMOOTH.MS Collected: 9/22/98 11:01 AM

Sample: 45



3/17

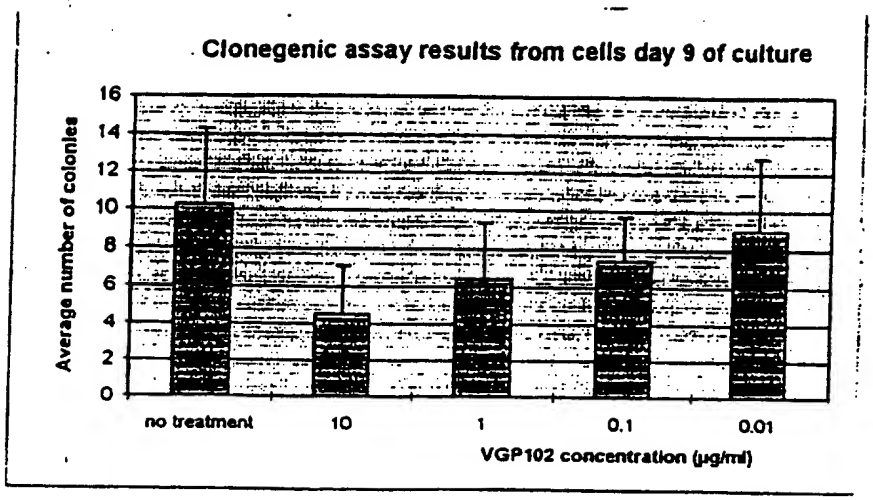
CA 02321256 2000-10-16

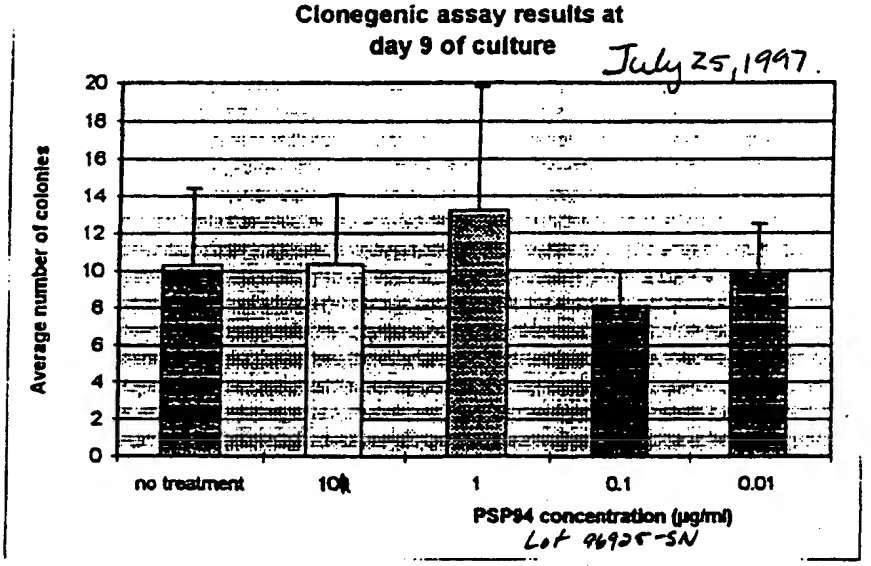
Fig. 3

Comment:

Method: LDE1008B	Accelerating Voltage: 20000	Laser : 1920	Low Mass Gate: OFF	Mirror Ratio:
Mode: Linear	Grid Voltage: 94.000 %	Scans Averaged: 27	Timed Ion Selector:	PSD Mirror Ratio:
	Guide Wire Voltage: 0.060 %	Pressure: 4.40e-07		
	Negative Ions: OFF			

Fig. 4

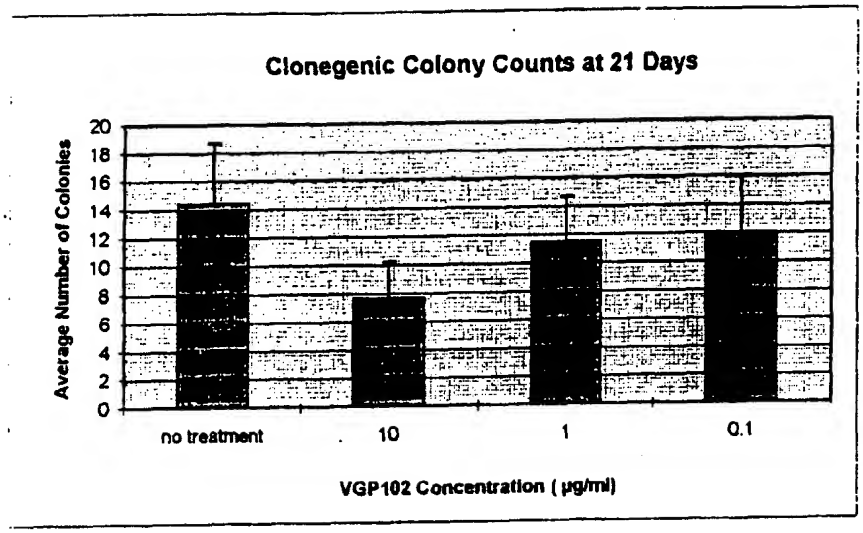


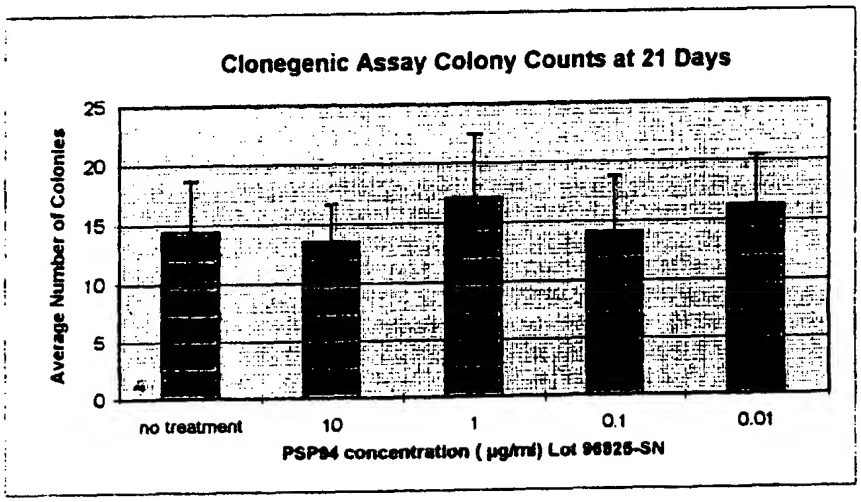


Cells seeded July 14

VGP102 = Decapeptide (Sequence ID No. 3)

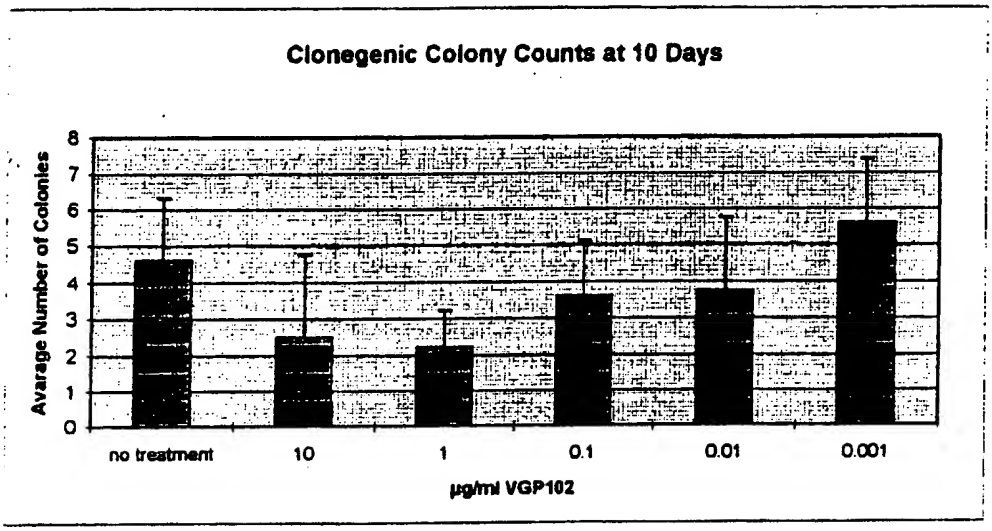
Fig. 5

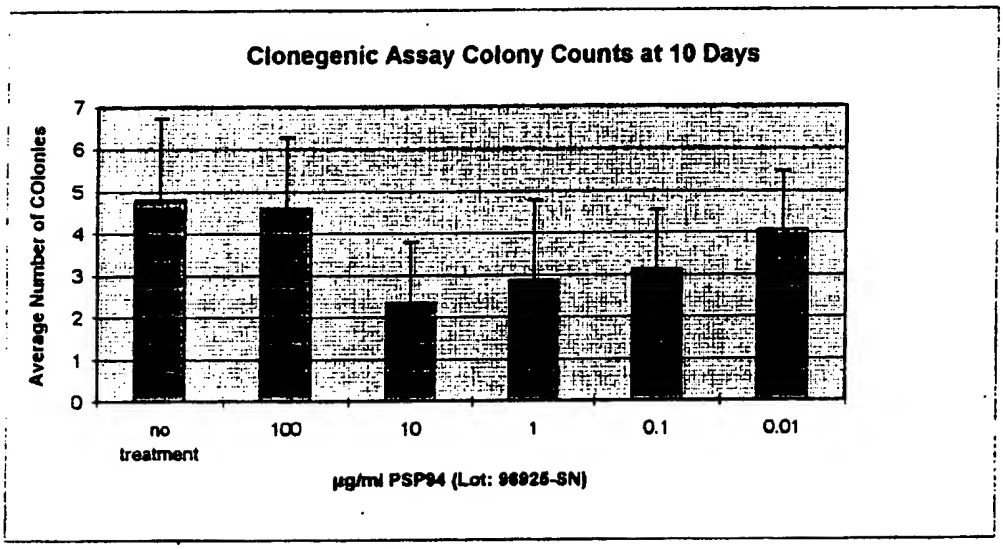




Cells seeded July 16.

Fig. 6

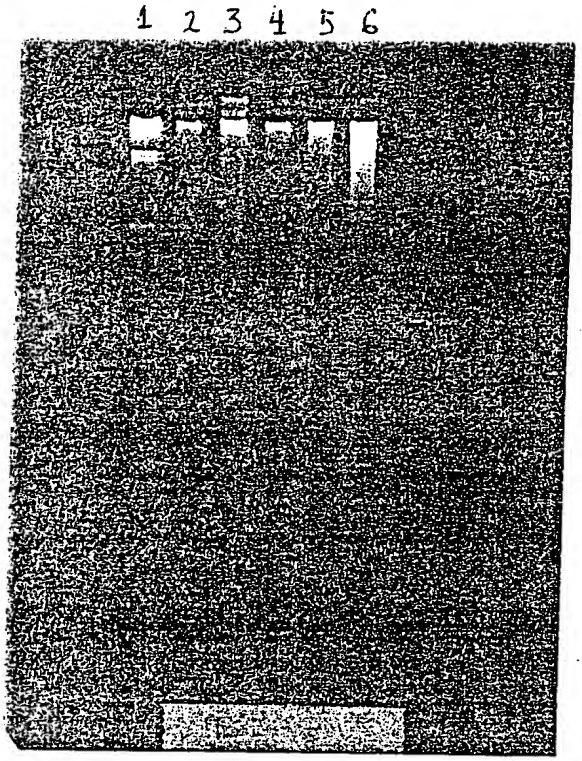




Seeded July 29, 1997

Fig. 7

1. Hind III marker
2. Control
3. 40μg native PSP94
4. 20μg " "
5. 22.5μM peptide 31-45
6. 45 μM peptide 31-45



DNA Fragmentation Following peptide 31-45 (Sequence ID No. 5) Treatment of PC-3 cells.

Apoptosis assay with Elisa plus kit following peptide treatment on atcc-PC3 cells for 72hrs

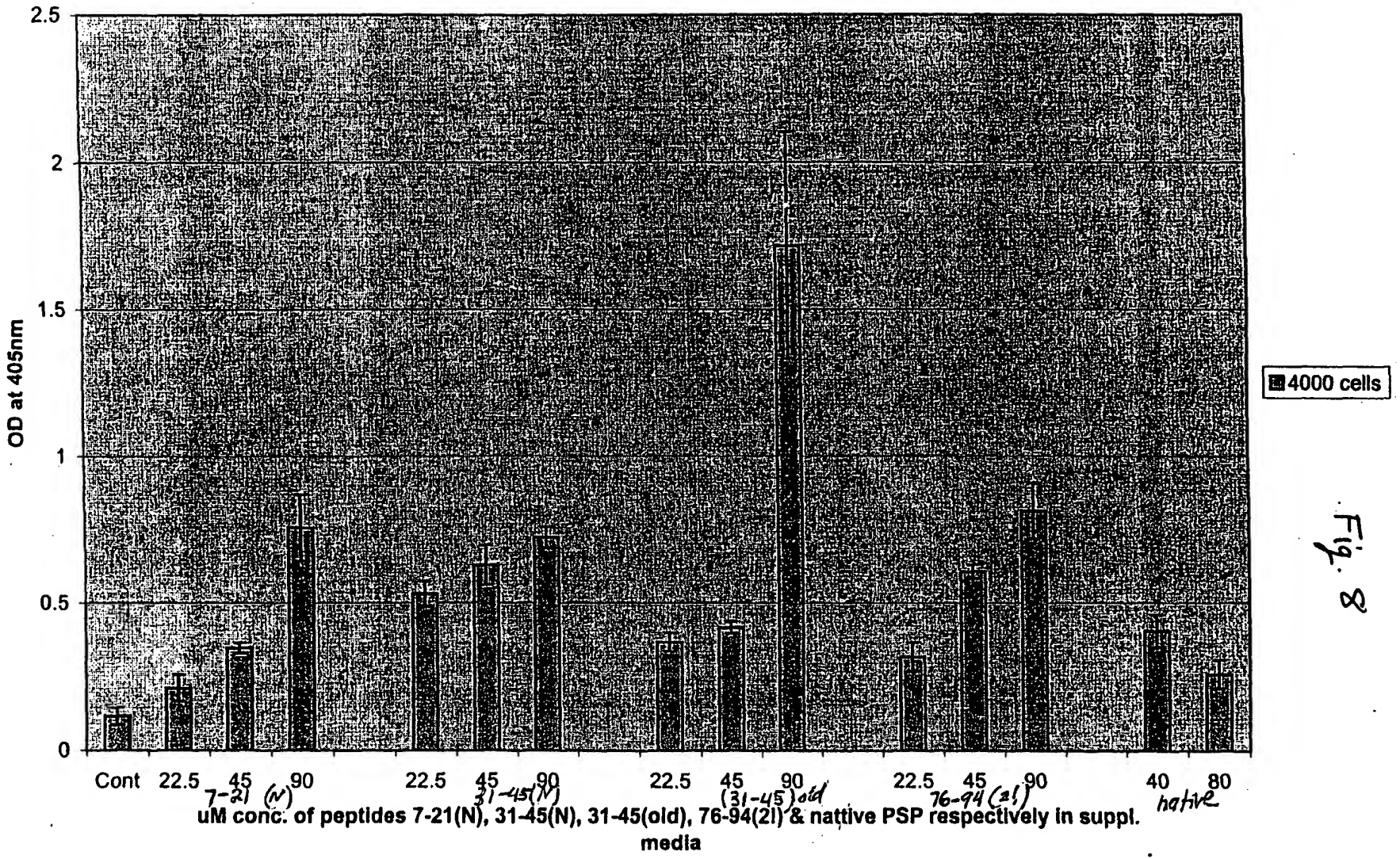


Fig. 8

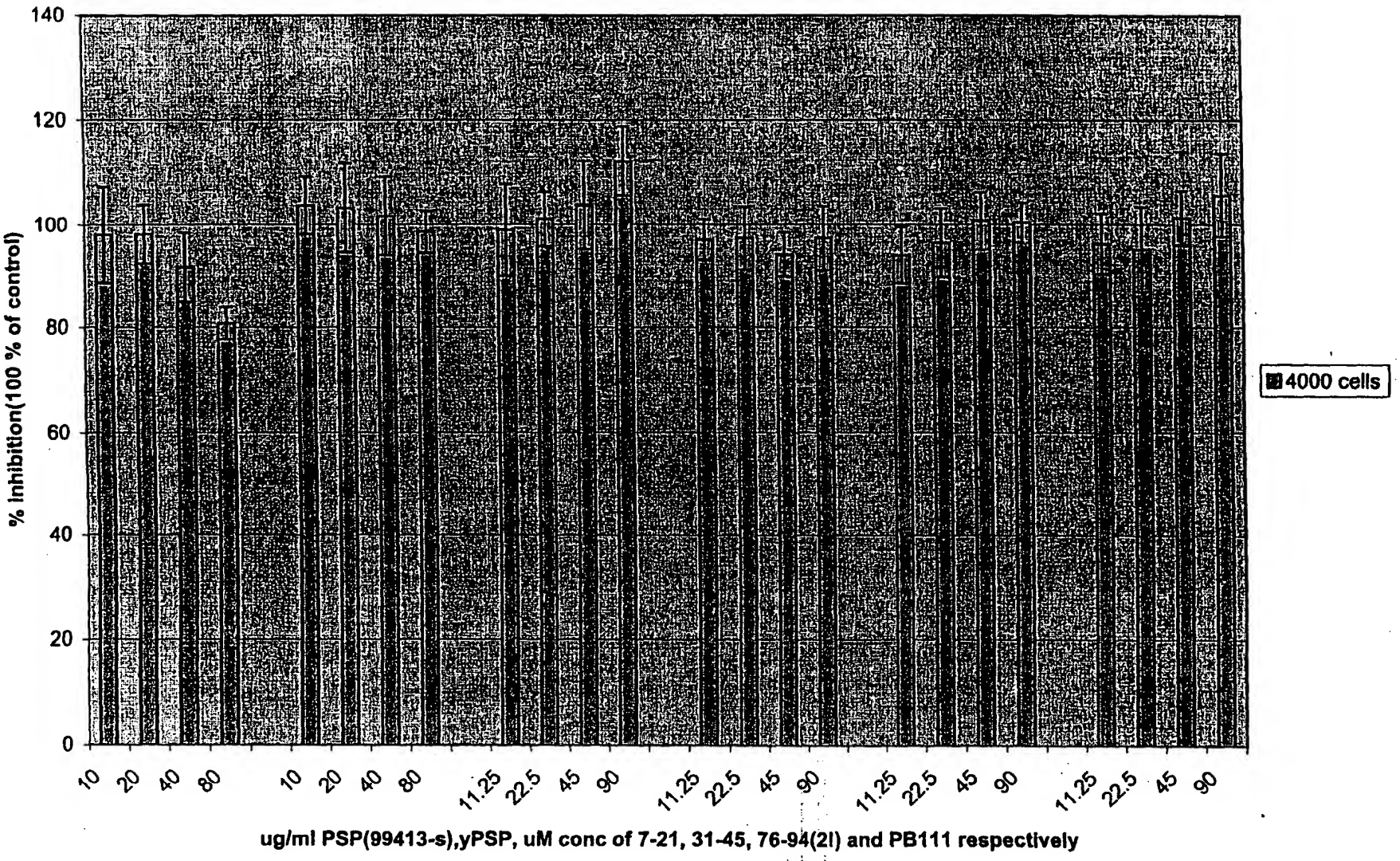

Growth of fibroblast cells when exposed to PSP and its analogues in vitro for 72hrs
% inhibition(100 % of control)
140
120
100
80
60
40
20
0
10
20
40
80
10
20
40
80
11.25
22.5
45
90
11.25
22.5
45
90
11.25
22.5
45
90
11.25
22.5
45
90
4000 cells
ug/ml PSP(99413-s),yPSP, uM conc of 7-21, 31-45, 76-94(2I) and PB111 respectively

Fig. 9

June 1, 1999.

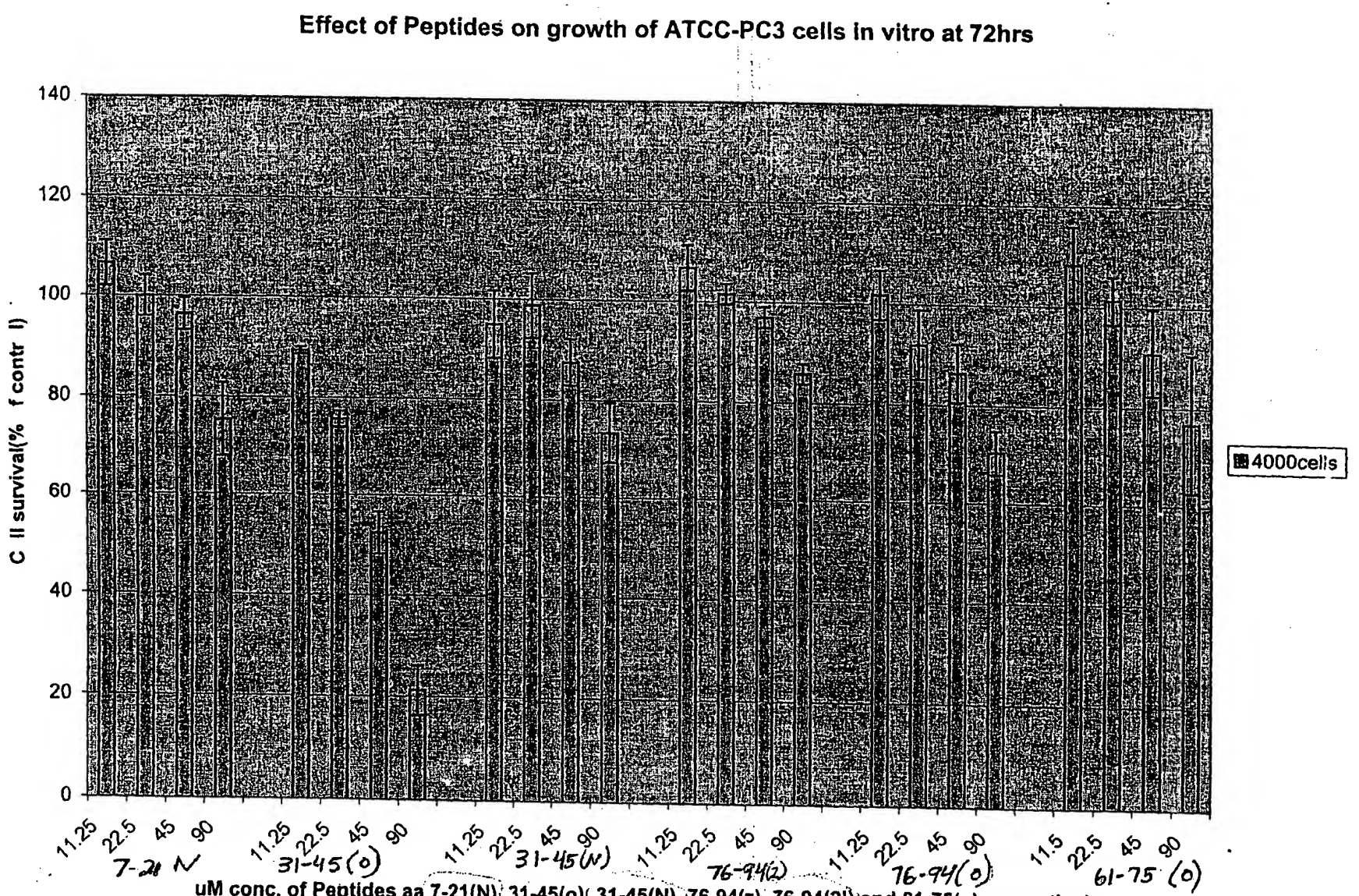


Fig. 10

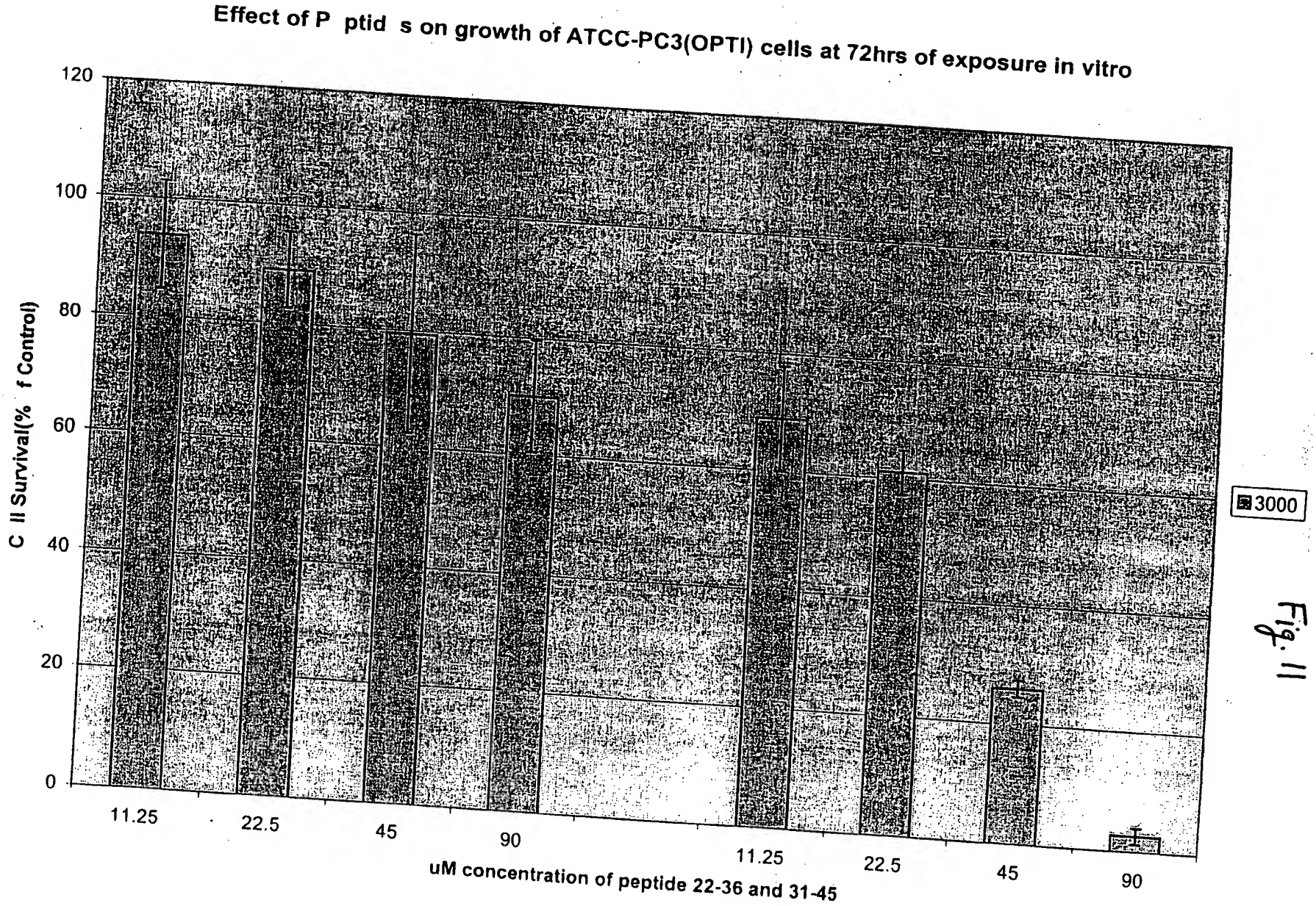

Effect of P ptid s on growth of ATCC-PC3(OPTI) cells at 72hrs of exposure in vitro
C ll Survival(% f Control)
120
100
80
60
40
20
0
11.25
22.5
45
90
11.25
22.5
45
90
uM concentration of peptide 22-36 and 31-45
3000

Fig. 11

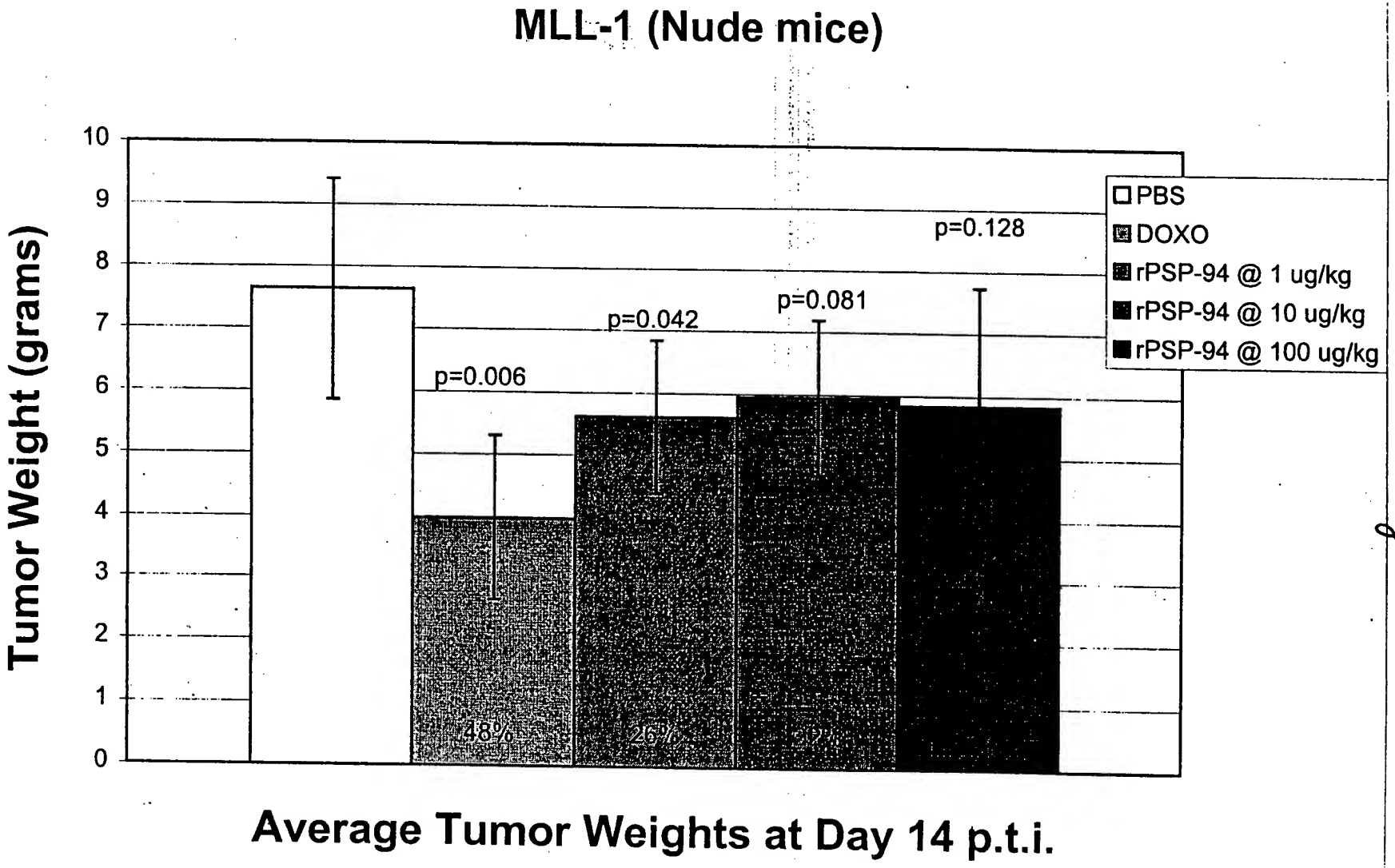

MLL-1 (Nude mice)
Tumor Weight (grams)
10
9
8
7
6
5
4
3
2
1
0
p=0.006
p=0.042
p=0.081
p=0.128
48%
26%
PBS
DOXO
rPSP-94 @ 1 ug/kg
rPSP-94 @ 10 ug/kg
rPSP-94 @ 100 ug/kg
Average Tumor Weights at Day 14 p.t.i.

Fig 12

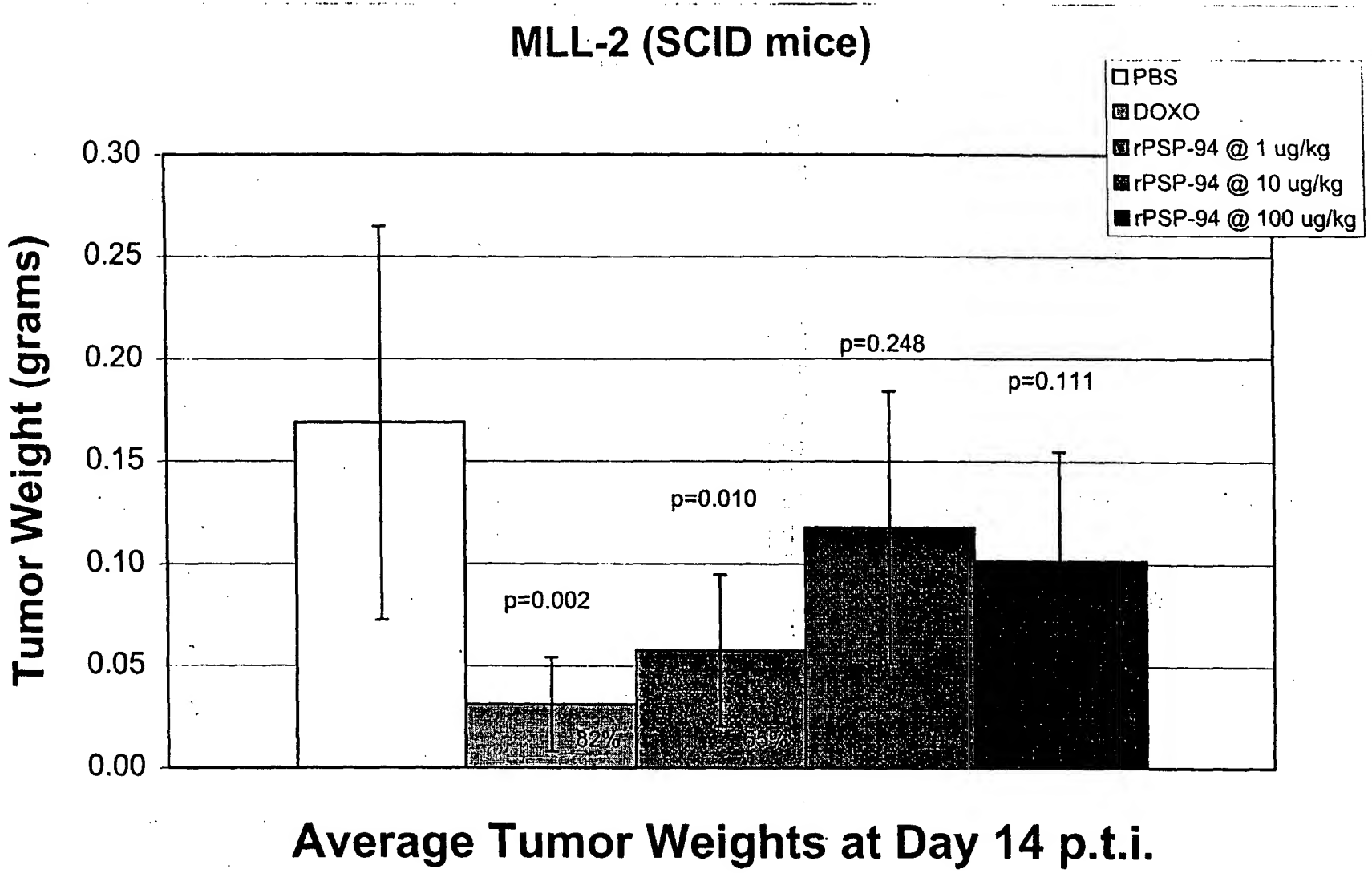

MLL-2 (SCID mice)
PBS
DOXO
rPSP-94 @ 1 ug/kg
rPSP-94 @ 10 ug/kg
rPSP-94 @ 100 ug/kg
Tumor Weight (grams)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
p=0.002
p=0.010
p=0.248
p=0.111
82%
Average Tumor Weights at Day 14 p.t.i.

Fig. 13

Fig. 14

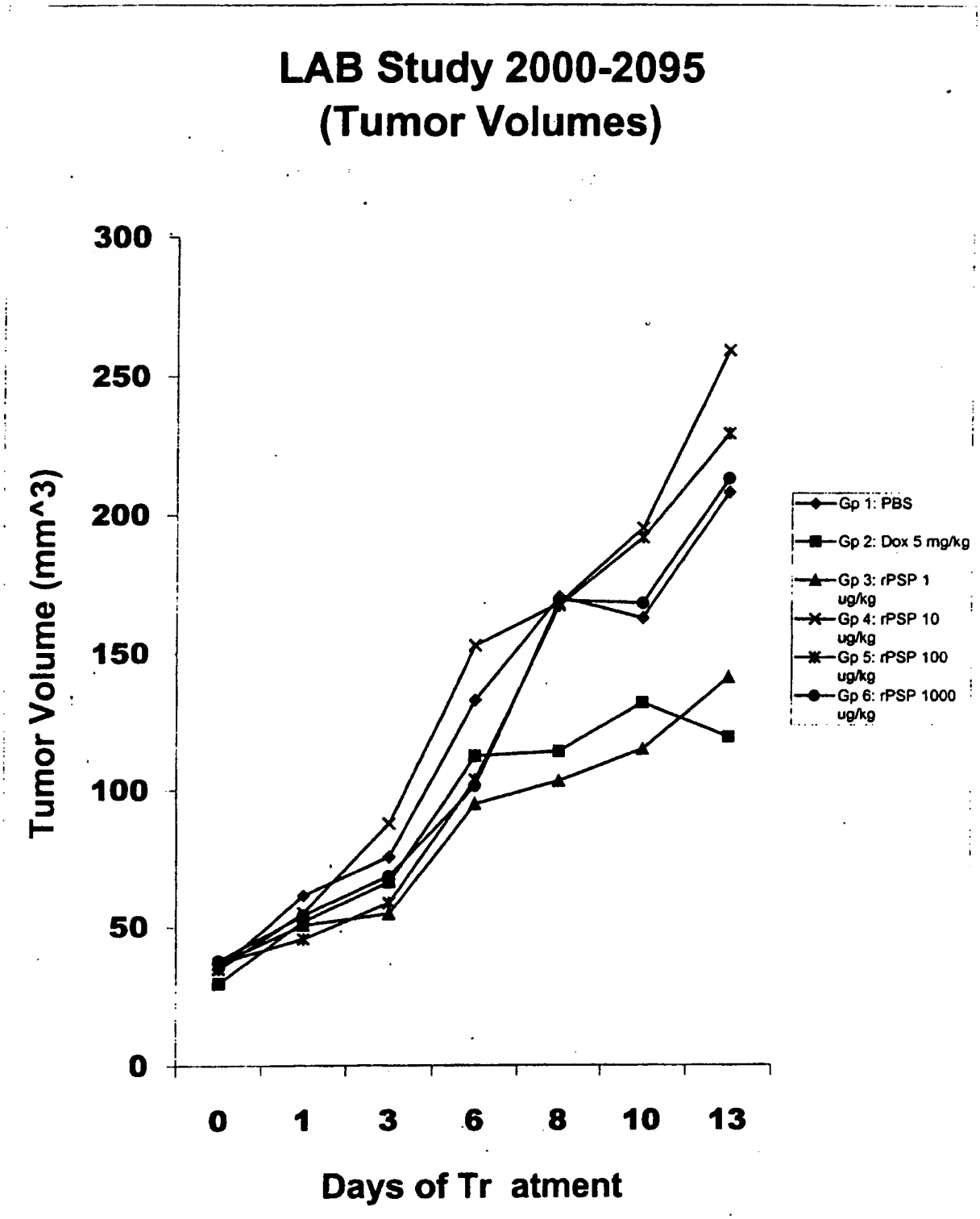
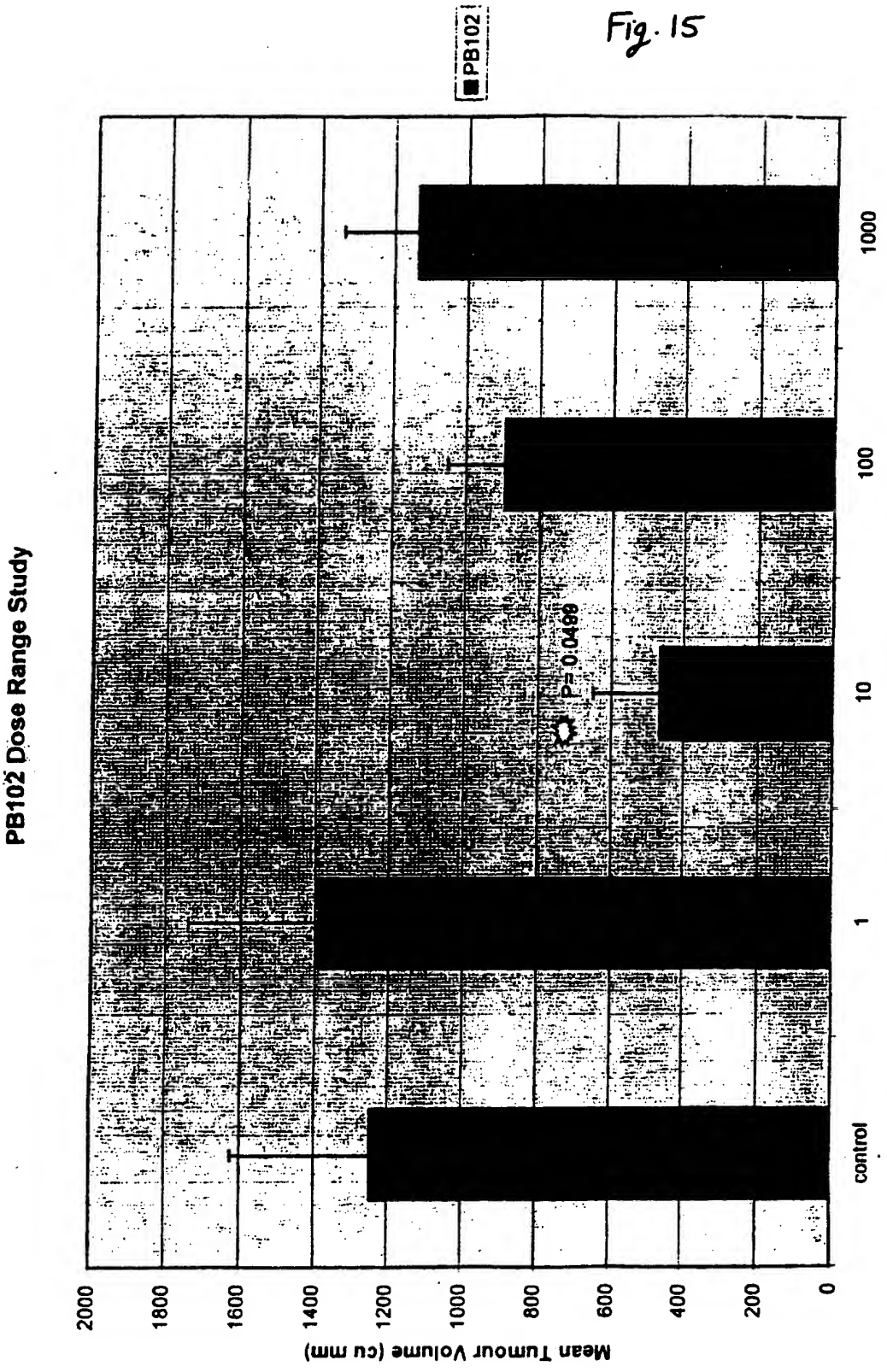
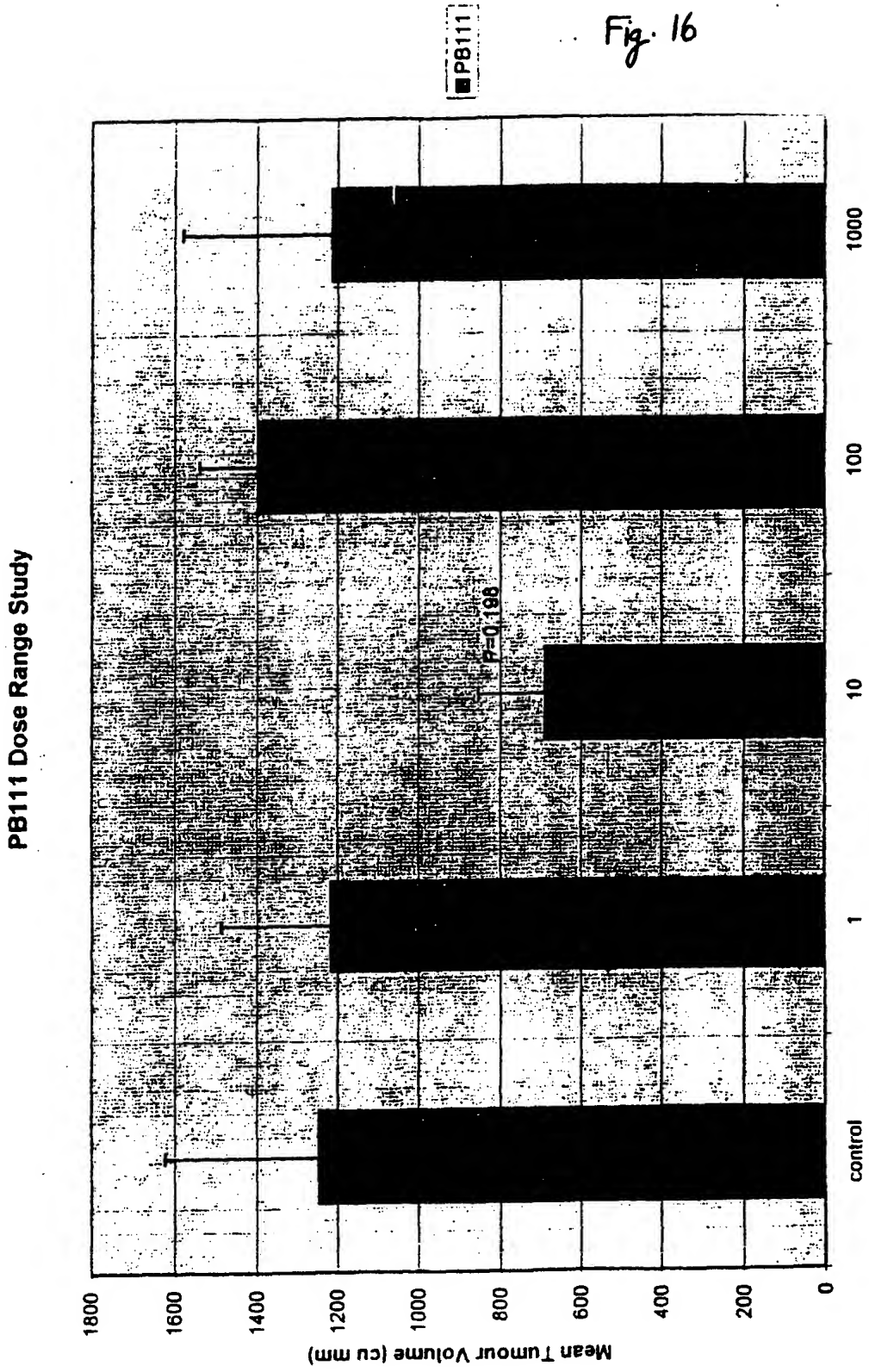

LAB Study 2000-2095
(Tumor Volumes)
Tumor Volume (mm^3)
300
250
200
150
100
50
0
0
1
3
6
8
10
13
Days of Tr atment
Gp 1: PBS
Gp 2: Dox 5 mg/kg
Gp 3: rPSP 1 ug/kg
Gp 4: rPSP 10 ug/kg
Gp 5: rPSP 100 ug/kg
Gp 6: rPSP 1000 ug/kg

Fig. 15



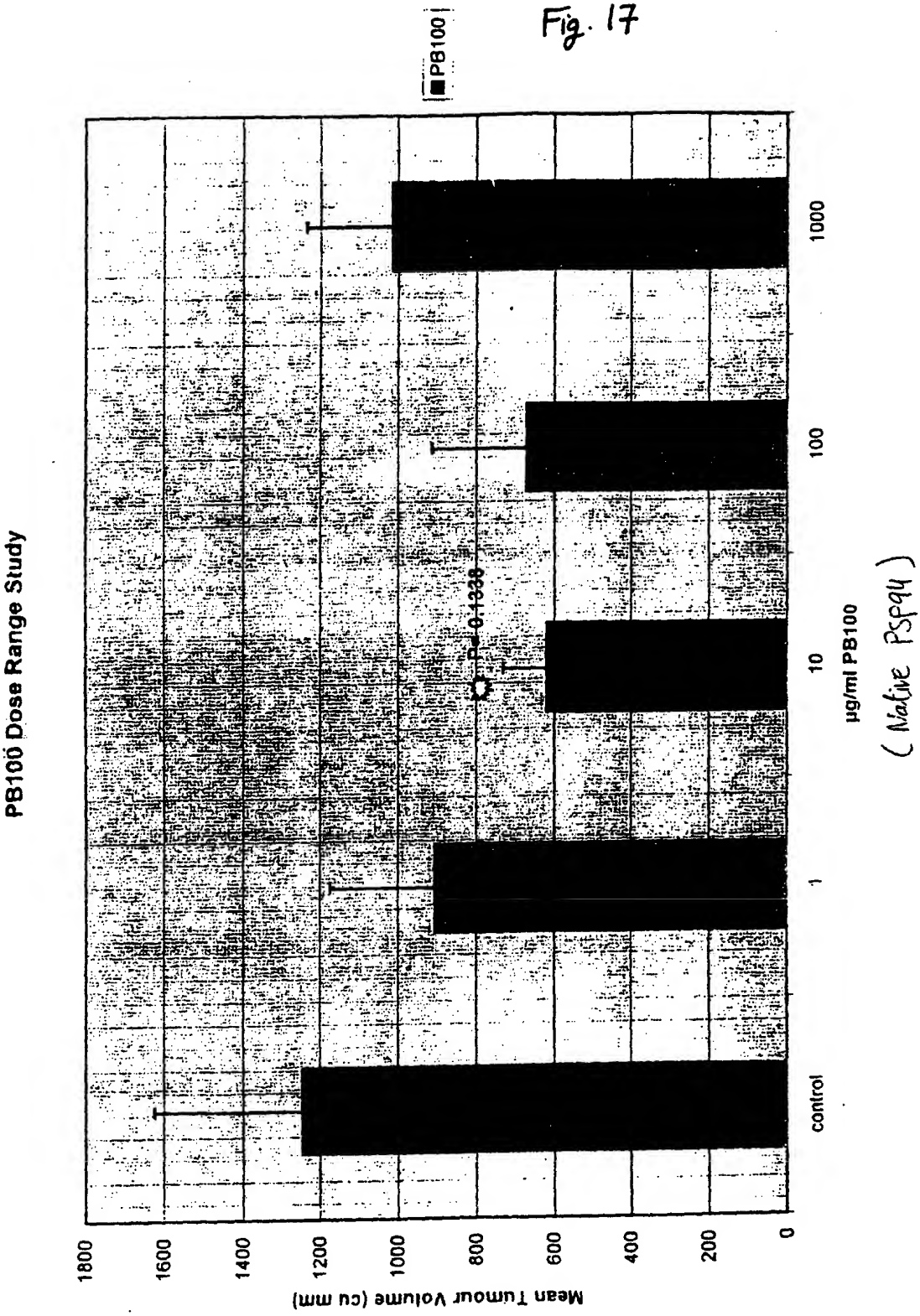
(Decapeptide, Sequence ID No. 3)

Fig. 16



(Control Decapeptide)

Fig. 17



(Native PSP94)